Immunological Reviews 1995, No. 144
Printed in Denmark . All rights reserved

Immunological Reviews
ISSN 0105-2896

Monoclonal anti-TNFα Antibody as a Probe of Pathogenesis and Therapy of Rheumatoid Disease

RAVINDER N. MAINI, MICHAEL J. ELLIOTT, FIONULA M. BRENNAN, RICHARD O. WILLIAMS, CONG QIU CHU, EWA PALEOLOG, PETER J. CHARLES, PETER C. TAYLOR & MARC FELDMANN

THE BURDEN OF RHEUMATOID DISEASE AND LIMITATIONS OF CURRENT THERAPY

Rheumatoid arthritis (RA) is a chronic disabling and painful disease of multiple joints which is prevalent in all parts of the world. Population studies suggest that around 1% of the population is affected, the disease being three times commoner amongst women than men. The peak incidence is in middle age but no age is exempt and it afflicts all age groups including children and the elderly. Although the disease spectrum is broad, ranging from mild to severe, the disabling effects are cumulative and, in the severe group, associated with premature death (Erhardt et al. 1989, Pincus & Callahan 1993).

Current management of RA is based around a multidisciplinary team with drug therapy playing a key role in alleviating symptoms, maintaining mobility and slowing the progression of joint damage. However, a great majority of patients require frequent changes in drug therapy due to loss of efficacy and/or to toxicity and the lack of significant control of disease becomes all too self-evident in the second decade from onset. Surgical treatment, including multiple joint prostheses, provides respite but highlights the failure of medical treatment. The economic, social and personal cost of RA is clearly unacceptable and motivates the search for better and more effective therapeutic and preventative measures.

Kennedy Institute of Rheumatology, Hammersmith, London, W6 7DW.
Correspondence to: Professor R. N. Maini, Kennedy Institute of Rheumatology, Bute Gardens, London, W6 7DW, UK.

NEW THERAPIES BASED ON TARGETING DISEASE MECHANISMS

Initiating factors and cellular targets

Attempts at discovery of effective novel cures for RA are based on the premise that knowledge of the causal factors and pathogenesis of RA has advanced significantly enough to construct hypotheses which define cellular and/or molecular targets. The etiology of RA is largely unknown, although population and family studies, together with observed concordance rates of disease in monozygotic twins, indicate that multiple germline genes are implicated with the genetic contribution to RA of 15% (Silman et al. 1993); however, a recent re-analysis has suggested that genetic factors could account for up to 60% of susceptibility (Macgregor & Silman 1994). The polymorphic HLA-DR β chain genes (some DR4 subtypes and DR1), encoding the 'shared epitope' pentapeptide sequence QRRAA, appears to be the main identified susceptibility gene (Gregersen et al. 1987). However, in the majority of patients a major role is attributed to non-genetic factors, and it is suggested that environmental factors, sex hormones and function of the hypothalamic-pituitary-adrenal axis, play a part (Reviewed in Silman & Hochberg 1993, Masi 1994, Chikanza et al. 1992). The association of RA with a HLA-DR molecule, which is involved in antigen binding and presentation to T cells, the presence in rheumatoid joints of activated memory T cells, and of autoantibodies in serum of patients, has suggested an important role for an immune response in initiating and maintaining RA. However, no convincing evidence for a rheumatoid specific antigen has been forthcoming, although antigens such as collagen II, abundant in cartilage, are possible candidates, and do elicit an antibody response in a majortity of DR4 positive RA patients (Ronnelid et al. 1994). Indeed we have documented a single RA patient in whom collagen II specific T-cell clones were repeatedly cloned from joints over a period of several years (Londei et al. 1989), but, in general, we can only demonstrate T-cell reactivity to collagen II antigens in a minority of RA patients. Alternatively, analysis of T-cell receptors in the joints in some studies has pointed to a restriction and selection bias which could suggest the involvement of superantigens (Paliard et al. 1991).

Since the involvement of T cells is central to the immunological paradigm, a great deal of attention has been paid to characterizing the prominent T-cell infiltrate in RA joints. The results of these investigations have led to the general conclusion that $CD4^+$ cells of the memory phenotype which bear activation markers are predominant (Panayi et al. 1992). Although there is a lack of consensus on the level of T cell cytokine expression, with the majority of investigators only finding low, but detectable, levels of interleukin-2 (IL-2) and interferon γ (IFNγ) at protein level (Firestein & Zvaifler 1990, Buchan et al. 1988a), there is an emerging consensus that there is a deficiency of IL-4 secretion in established disease (Moissec et al. 1990, Simon et al. 1994). However, at mRNA level IFNγ

is consistently detectal
a predominance of Th
compatible with a T c

These concepts of th
approaches, summariz
lating sex hormones o
ic-pituitary-adrenal ax
which effectively 'bloc
pation by a putative R
that collagen II is an i
immunodominant or c
temic administration o
strations that with sub
are converted into ant

Targetin

Target
Sex hormones
Hypothalamic-pituitary-adrenal axis
HLA DR4/1
Antigen
Superantigen
Th1 cells
T cells
Gut associated lymphoid system

MECHANISMS

ased on the premise
A has advanced sig-
lar and/or molecular
opulation and family
ease in monozygotic
ith the genetic contri-
t re-analysis has sug-
susceptibility (Mac-
in genes (some DR4
ntapeptide sequence
ene (Gregersen et al.
is attributed to non-
rs, sex hormones and
part (Reviewed in Sil-
he association of RA
ding) presentation
emory T cells, and of
ortant role for an im-
r, no convincing evi-
ng, although antigens
didates, and do elicit
patients (Ronnelid et
in whom collagen II
er a period of several
monstrate T-cell reac-
Alternatively, analysis
d to a restriction and
erantigens (Paliard et

nological paradigm, a
prominent T-cell infil-
ve led to the general
which bear activation
ere is a lack of consen-
ajority of investigators
IL-2) and interferon γ
i et al. 1988a), there is
ecretion in established
, at mRNA level IFNγ

is consistently detectable (Buchan et al. 1988a, Cohen et al. in press), suggesting a predominance of Th1 over Th2 subsets of T cells (Mossman & Coffman 1989), compatible with a T cell driven chronic inflammatory disease.

These concepts of the etiopathogenesis of RA have suggested many therapeutic approaches, summarized in Table I. These range from proposals that manipulating sex hormones or supplementing corticosteroids to restore the hypothalamic-pituitary-adrenal axis may be beneficial, to seeking synthetic designer peptides which effectively 'block' the HLA antigen binding site thus preventing its occupation by a putative RA specific autoantigen. For those investigators convinced that collagen II is an important autoantigen, attempts are being made to define immunodominant or cryptic epitopes that might induce tolerance either by systemic administration or via the oral route (Trentham et al. 1993). Recent demonstrations that with substitutions of one or two amino acids, immunogenic peptides are converted into antagonists, which result in immunological unresponsiveness

TABLE I

Targeting initiating factors and the T cell response in RA

Target	Observation	Possible therapy
Sex hormones	Female preponderance Oral contraceptives protective	Anti-oestrogens, male sex hormones Progesterones
Hypothalamic-pituitary-adrenal axis	Disturbed, poor corticosteroid response to surgical stress	Early use of corticosteroids
HLA DR4/1	Association of 'shared epitope' with severe RA	Peptide blockade
Antigen	B and T cell collagen II reactivity	Induce tolerance by altered peptide ligands or soluble immunodominant peptides
Superantigen	Restricted TcR usage with CDR3 diversity	Induce tolerance with superantigens
Th1 cells	Predominant Th1 cell in joints is pro-inflammatory; IL-4, IL-12 anti-inflammatory and deviate to protective Th2 response	IL-4 (IL-10) therapy
T cells	Activated, $CD4^+$ memory cells enriched in synovium	IL-2 diphtheria toxin conjugate and monoclonal antibody therapy, e.g., anti-IL-7, anti-CDW52, anti-CD4, anti-CD5
Gut associated lymphoid system	Oral collagen II induces bystander suppression via TGF-β producing cells trafficking to joints	Oral collagen feeding

or induce secretion of anti-inflammatory cytokines (e.g., IL-4, IL-10), have raised hopes that peptide therapy may prove useful in RA (Evavold et al. 1993). Peripheral tolerance, under certain circumstances, can also be induced by superantigens (O'Heir & Lamb 1990). Other manipulations with regulatory cytokines can convert a predominantly pro-inflammatory autoimmune Th1 response to an anti-inflammatory Th2 response, for example by administration of IL-4, with beneficial results (Racke et al. 1994).

Whilst many of these ideas are in early stages of development, T cell targeted therapies have already been used in clinical trials in RA. The major therapeutic modality has been the use of monoclonal murine and chimeric anti-CD4 antibodies on several hundred patients; additionally, a monoclonal antibody anti-CD5-toxin, murine anti-CD7 monoclonal antibody, a humanized anti-CDW52 monoclonal antibody (CAMPATH-1H) and an IL-2 diphtheria fusion toxin have all been used in clinical trials (reviewed by Elliott & Maini 1994). However, encouraging data from early open-label studies have not withstood more rigorous randomized placebo-controlled trials with disappointing results reported for anti-CD4, anti-CD5 immuno-conjugate and IL-2 diphtheria toxin fusion proteins (Moreland et al. 1993a, Van der Lubbe et al. 1994, Olsen et al. 1994, Moreland et al. 1993b). Progress with further testing of the promising results with CAMPATH-1H (Isaacs et al. 1992) has been set back by reports of susceptibility to virus and bacterial infections in treated patients.

Attempts at inducing antigen-specific regulatory T cells have been put to clinical trial by daily oral feeding of collagen type II, a constituent of cartilage, to RA patients in order to induce 'by-stander' suppression of activated T cells in joints (Trentham et al. 1993). In this approach it is speculated that oral xenogeneic collagen II activates a population of immunoregulatory gut associated lymphoid cells, which home to the joints, and there receive further activation signals from antigen-presenting cells which have locally processed collagen II from damaged cartilage. Here, anti-inflammatory cytokines such as TGF-β produced by the Th2, lymphocytes originating from gut, suppress pro-inflammatory Th1 cells (Chen et al. 1994). Promising results have been reported in early trials with some patients showing remission of their RA.

Cytokines and anti-cytokines

In our research, we have focused our attention on cytokines as promising therapeutic targets, since in the past decade impressive progress has been made in isolating a number of cytokines and their cognate receptors, and interactions between them which mediate the biological and pharmacological functions relevant to the pathogenesis of RA (Feldmann et al. 1993). For example, cytokines provide a framework for understanding how circulating leukocytes might adhere to and migrate into inflammatory sites, of which the RA joint is an example. At

these inflammatory si
cellular interaction vi
in sustaining chronic
orchestrate interactio
tive tissue cells and m
lage and bone, with i

In terms of specific
latoratories, including
are abundantly expre
even in patients recei
expressed cytokines j
which exert pleiotropi
the joint pathology o
clude induction of ad
molecule (ICAM-1) a
mote cell migration a
flammatory and tissu
ases (Dayer et al. 198
function, such as acti
of anabolic activity o
tion of cartilage and
locally produced cyto
es, such as IL-8 (Bren
polymorphs and mac
vates granulocytes an
local effects, such as
production, all of wh
duced and is importa
et al. 1988). Its local a
tion of antibodies (he
position consistent wi
IL-6 antibody (Wendl
ual cytokines found
and IL-6 might be ex
of developing specific
task, and fortunately
infra).

An appealing alter
duction of naturally
RA inflammation is
interleukin-1 receptor
the specific inhibitors

these inflammatory sites cytokines regulate growth, differentiation, cell death and cellular interaction via autocrine and paracrine activities which are implicated in sustaining chronic immune and inflammatory reactions. Here cytokines also orchestrate interactions between the immigrant cells and locally resident connective tissue cells and matrix to produce adherent pannus tissue, which erodes cartilage and bone, with incomplete attempts at regeneration and repair.

In terms of specific cytokines found in RA joints, research from a number of latoratories, including ours, leads to the conclusion that many of the cytokines are abundantly expressed during both the chronic and acute stages of disease, even in patients receiving 'optimal' anti-rheumatoid therapy. These abundantly expressed cytokines include IL-1α, IL-1β and tumor necrosis factor (TNFα) which exert pleiotropic, overlapping and synergistic biological effects relevant to the joint pathology of RA (Buchan et al. 1988b). Relevant biological effects include induction of adhesion molecules such as E-selectin, intercellular adhesion molecule (ICAM-1) and vascular cell adhesion molecule (VCAM-1) which promote cell migration and adhesion in joints (Bevilacqua 1993), induction of inflammatory and tissue-damaging molecules such as prostaglandins and collagenases (Dayer et al. 1985); nitric oxide (Palmer et al. 1993); and effects on cellular function, such as activation of osteoclastic resorption of bone, and suppression of anabolic activity of chondrocytes and osteoblasts, thereby impairing regeneration of cartilage and bone (Saklatavala et al. 1985, Gowen et al. 1983). Other locally produced cytokines with relevant pro-inflammatory activity are chemokines, such as IL-8 (Brennan et al. 1990b) and RANTES (Schall 1991), which attract polymorphs and macrophages to joints; and GM-CSF which mobilizes and activates granulocytes and macrophages from the bone marrow, as well as mediating local effects, such as upregulating HLA Class II expression and interleukin-1 production, all of which ultimately promote inflammation. IL-6 is also over-produced and is important in inducing acute phase proteins by hepatocytes (Hirano et al. 1988). Its local action on differentiation of B cells may contribute to production of antibodies (hence immune complexes) which are pro-inflammatory, a proposition consistent with clinical benefit attributed to the use of intravenous anti-IL-6 antibody (Wendling et al. 1993). Therefore, blocking the activity of individual cytokines found in RA joints, i.e. IL-1, TNFα, IL-8, RANTES, GM-CSF and IL-6 might be expected to result in anti-rheumatoid effects, but the prospect of developing specific molecular inhibitor drugs for each cytokine is a daunting task, and fortunately subsequent events have shown it to be unnecessary (*vide infra*).

An appealing alternative therapeutic approach is based on exploiting the production of naturally occurring inhibitors of cytokines produced in RA joints. RA inflammation is characterized by enhanced, but insufficient, production of interleukin-1 receptor antagonist (IL-1ra) and soluble TNF receptors, which are the specific inhibitors of IL-1 and TNFα respectively (Deleuran et al. 1992a, Cope

et al. 1992), and of IL-10 (Katsikis et al. 1994), which inhibits synthesis of IL-1 and TNFα. Augmenting inhibitor production *in situ* or supplementing inhibitor by injecting recombinant soluble receptors, usually as multimeric-immunoglobulin fusion proteins, in order to increase affinity and biological half-life *in vivo*, provide further scope for anti-cytokine drug development.

PIVOTAL ROLE OF TNFα

Our hypothesis that TNFα is a potential target for therapeutic intervention in RA was derived from experiments performed *in vitro* on RA synovial tissue. These studies indicated that dissociated synovial tissue mononuclear cells (MNCs) from RA patients were highly activated, and if placed in culture would produce cytokines spontaneously for 5–6 days without exogenous stimulation. The cytokines which are produced in abundance include IL-1α and IL-1β (Buchan et al. 1988b), TNFα (Brennan et al. 1989), IL-6 (Hirano et al. 1988), IL-8 (Brennan et al. 1990b), TGFβ (Brennan et al. 1990a), GM-CSF (Haworth et al. 1991) and, as shown more recently, IL-10 (Katsikis et al. 1994). As IL-1 and TNFα have pro-inflammatory potential implicated in the pathogenesis of RA, we focused on the regulation of these cytokines.

Using a polyclonal anti-TNF antibody to block TNFα activity in the RA synovial cell cultures, we observed that this also resulted in the inhibition of IL-1 production (Brennan et al. 1989). The result suggested the presence of a 'cytokine cascade' within the synovium, with the prediction that blockade of a pivotal cytokine in this network would subsequently lead to blockade of other cytokines 'downstream'. This indeed was found to be the case in that the production of other pro-inflammatory cytokines including GM-CSF (Haworth et al. 1991), IL-6 and IL-8 (Butler et al. submitted) was also modulated by TNFα blockade. The biological effect of TNFα is enhanced further by our observation that both p55 and p75 surface TNF receptors are upregulated on RA synovial tissue cells (Brennan et al. 1991, Deleuran et al. 1992b) (Fig. 1). Indeed, CD68 positive macrophages at the cartilage pannus junction (Chu et al. 1992) also express abundant p55 and p75 TNF-R, indicating the potential of these cells to respond in an autocrine manner. The biological activity of TNFα is regulated to some degree, however, by the production of its native inhibitors soluble p55 and p75 TNF-R. These soluble TNF-R are also found in elevated amounts in RA plasma and in particular, in synovial fluid where their presence in excess neutralizes TNFα activity (Cope et al. 1992). The production of sTNF-R is insufficient however, as bioactive TNFα was found in all RA synovial MNC cultures tested.

A second immunoregulatory molecule, IL-10, is also abundantly produced by RA synovial MNC cultures (Katsikis et al. 1994). Endogenous IL-10 was found to be functional as its neutralization enhanced TNFα and IL-1 production 2–3 fold (Katsikis et al. 1994), indicating that as in the production of other cytokine

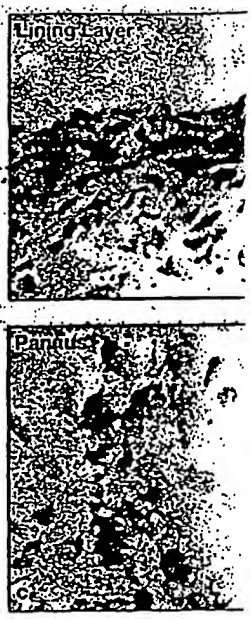


Figure 1. TNFα and p55 T
lage-pannus junction in a
(a) and TNF-R (b); simil
cartilage-pannus junction.

inhibitors including sT
1992a) homeostatic me
in insufficient quantitie
ological changes contin

EVIDENCE FOR TH

Murine collagen-induc
for human rheumatoi
similarities between th
pannus formation and
dition, susceptibility tc
MHC class II genes, s
both diseases is the MI
1989).

The CIA model has l
to human disease and

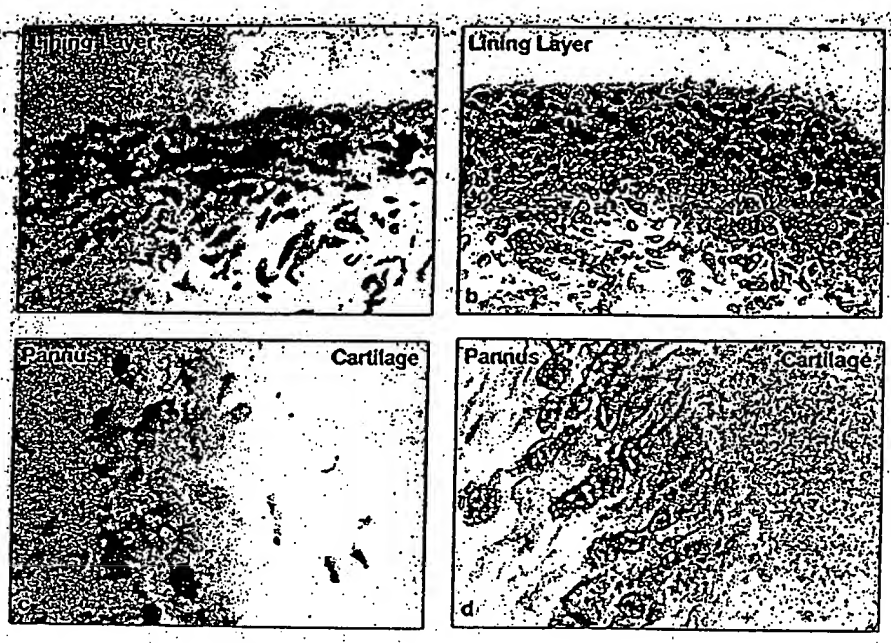


Figure 1. TNFα and p55 TNF-R demonstrated by immunohistology of synovium and cartilage-pannus junction in a RA joint. Majority of cells in synovial layer co-stain for TNFα (a) and TNF-R (b); similar co-localization of TNFα (c) and TNF-R (d) is observed at cartilage-pannus junction.

inhibitors including sTNF-R (Cope et al. 1992) and the IL-1ra (Deleuran et al. 1992a) homeostatic mechanisms are present in inflammatory environments, albeit in insufficient quantities, since cytokine production and the consequential pathological changes continue to occur.

EVIDENCE FOR THE IMPORTANCE OF TNFα FROM ANIMAL MODELS

Murine collagen-induced arthritis (CIA) has been extensively studied as a model for human rheumatoid arthritis (RA), principally because of the pathological similarities between the two diseases, including similar patterns of synovitis, pannus formation and erosion of cartilage and bone (Stuart et al. 1984). In addition, susceptibility to both human RA and murine CIA is linked to specific MHC class II genes, suggesting that an important step in the development of both diseases is the MHC-restricted activation of $CD4^+$ T cells (Holmdahl et al. 1989).

The CIA model has been used to elucidate pathogenic mechanisms of relevance to human disease and to identify potential targets for therapeutic intervention.

One of the methods used in such studies involves the administration of potentially pathological cytokines to naive mice or to mice previously immunized with type II collagen in order to demonstrate altered expression of disease. For example, the local administration, in recombinant form, of IFN-γ, IL-1β, or TNF to type II collagen-immunized mice has been shown to result in accelerated onset and increased severity of CIA, suggesting that these cytokines are involved in the induction of arthritis (Mauritz et al. 1988, Hom et al. 1988a, Thorbecke et al. 1992, Cooper et al. 1992, Brahn et al. 1992). None of the cytokines, however, was capable of initiating arthritis when injected into unimmunized mice, indicating that they could not, on their own, elicit a sustained inflammatory response. However, the experimental approach adopted in these studies has a number of limitations, including the fact that cytokines generally have extremely short half-lives *in vivo*.

An alternative approach would be to use transgenic mice to study the pathological effects arising from the sustained over-expression of cytokine transgenes. This approach powerfully demonstrated the arthritogenic potential of over-expression of TNFα in the joints of 3′-modified human TNFh-transgenic mice, which spontaneously develop a chronic arthritis that is prevented by anti-TNF treatment (Keffer et al. 1991). Our histopathological studies on the Kolias hTNF-transgenic mice show that the joints of these mice exhibit similar inflammatory lesions to those found in human RA, including proliferative synovitis and pannus formation. Most importantly, necrotic chondrocytes are oberved in the joints of hTNF-transgenic mice as well as severe focal erosions of sub-chondral bone (Fig. 2). These findings clearly show that the dysregulated expression of TNF is alone capable of inducing many of the pathological changes that are seen both in human RA and murine CIA, including full-blown destruction of cartilage and bone.

An alternative to the study of cytokines that contribute to the pathogenesis of arthritis is to treat collagen-arthritic mice or rats with specific anti-cytokine agents in order to show a reduction in disease severity. Following the identification of pro-inflammatory cytokines, in particular TNF and IL-1, in the joints of RA patients (Chu et al. 1991, Chu et al. 1992), we and others set out to address the question of whether the neutralization of such cytokines would lead to amelioration of autoimmune arthritis. A number of studies focused on the effect of anti-TNF treatment in CIA, partly because of data accumulated in our laboratories suggesting that, in RA at least, TNF was playing an important role in the induction of other pro-inflammatory cytokines. In CIA, two studies showed that monoclonal or polyclonal anti-TNF antibodies, administered before the onset of clinical arthritis, protected against the subsequent development of disease (Thorbecke et al. 1992, Piguet et al. 1992). Similarly, soluble TNF receptors, which are thought to serve as endogenous regulators of TNF activity, also protected against CIA when given during the pre-arthritic period (Piguet et al. 1992).

Our own research has focused largely on the effects of anti-TNF treatment in

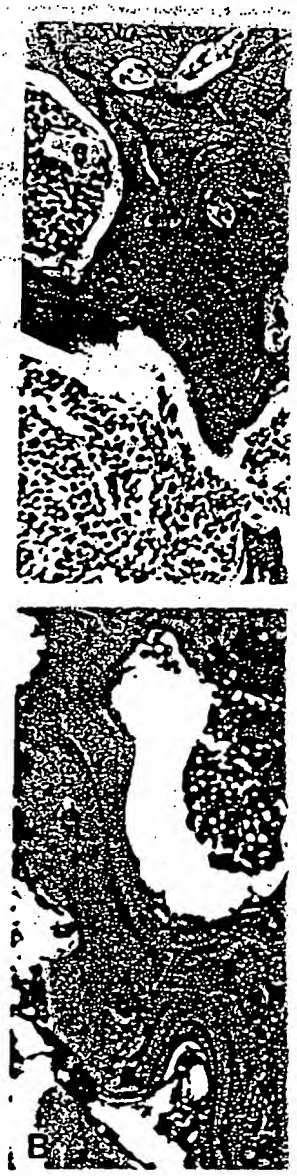


Figure 2. A: Light micrograp
from a hTNF-transgenic mou
pannus and loss of chondroc
transgenic littermate with cho

inistration of potentially
sly immunized with type
of disease. For example,
, IL-1β, or TNF to type
in accelerated onset and
ines are involved in the
1988a, Thorbecke et al.
: cytokines, however, was
nunized mice, indicating
nmatory response. How-
:s has a number of limi-
:xtremely short half-lives

ce to study the pathog-
:ytokine transgenes. This
ential of over-expression
sgenic mice, which spon-
by -TNF treatment
Kollias hTNF-transgenic
inflammatory lesions to
vitis and pannus forma-
:d in the joints of hTNF-
-chondral bone (Fig. 2).
ression of TNF is alone
hat are seen both in hu-
on of cartilage and bone.
te to the pathogenesis of
th specific anti-cytokine
llowing the identification
IL-1, in the joints of RA
:rs set out to address the
s would lead to amelior-
ised on the effect of anti-
lated in our laboratories
portant role in the induc-
udies showed that mono-
before the onset of clin-
:nt of disease (Thorbecke
IF receptors, which are
ty, also protected against
et al. 1992).
of anti-TNF treatment in

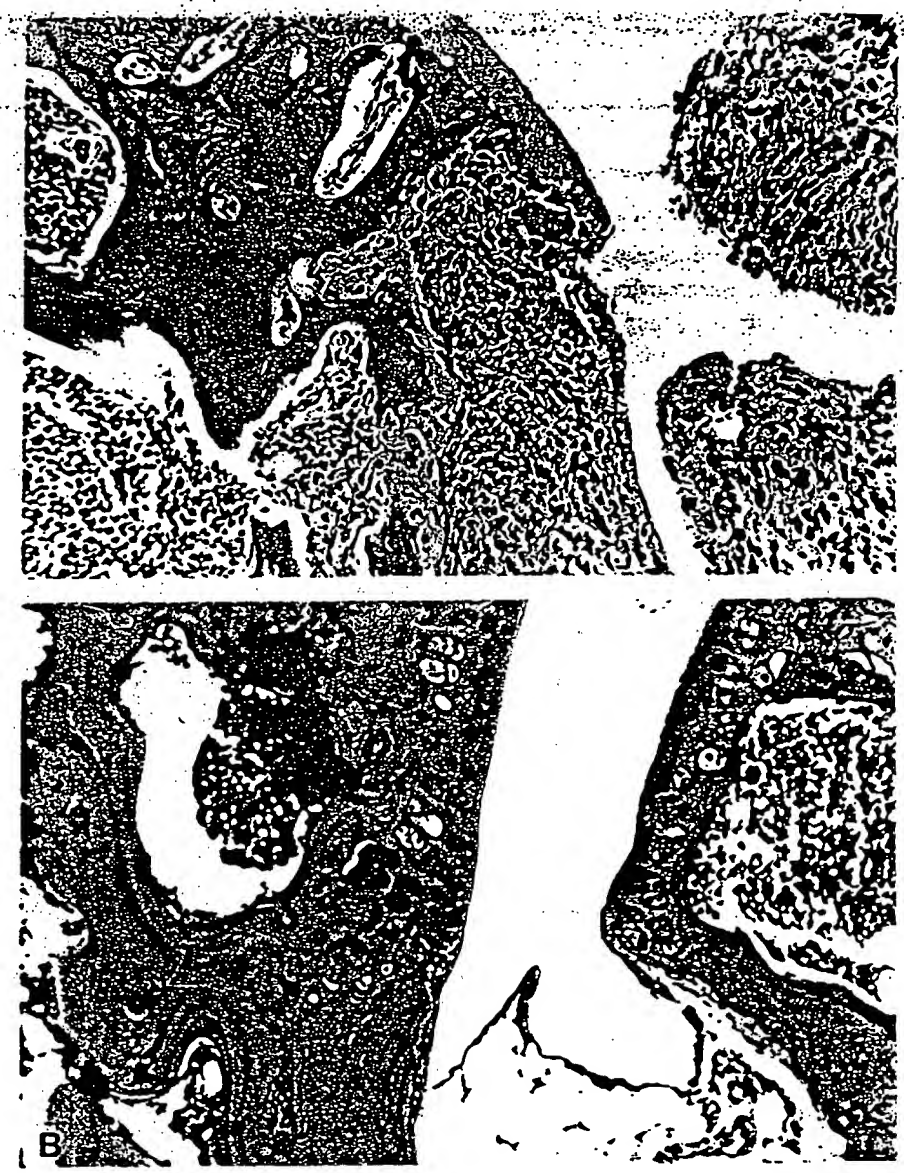


Figure 2. A: Light micrograph showing the cartilage-bone-pannus region of a knee joint from a hTNF-transgenic mouse. Note the focal erosion of sub-chondral bone by cellular pannus and loss of chondrocytes in cartilage. B: Normal joint and surfaces from non-transgenic littermate with chondrocyte-rich cartilage and normal bone.

established CIA, because of the greater significance of such studies in terms of human therapy. We were able to show that a hamster IgG1 anti-TNF monoclonal antibody (mAb) (TN3-19.12) administered over a 10-day period, starting immediately after the onset of clinically-detectable arthritis, caused a reduction in clinical score and suppressed paw-swelling, without altering circulating levels of anti-type II collagen IgG (Williams et al. 1992). In addition, histological analysis showed that anti-TNF treatment resulted in a significant reduction in joint erosion relative to control arthritic mice, as determined by the proportion of proximal-interphalangeal (PIP) joints showing erosive changes (Table I).

The long-term administration to man of anti-TNF mAbs containing epitopes of murine origin may be limited by the development of a neutralizing antibody response (Waldmann 1991). A TNF receptor-IgG fusion protein (of entirely human origin but containing neo-epitopes at the junction of IgG and TNF receptor) may, therefore, provide a less immunogenic alternative to mAbs, provided that the fusion protein can be shown to be effective *in vitro*. In one study, a human p75 TNF receptor-Fcγ fusion protein was found to reduce the clinical severity of CIA, whether administered before or after the onset of arthritis (Wooley et al. 1993a). We were subsequently able to confirm the clinical efficacy of this form of treatment in established CIA using a human p55 TNF receptor-IgG fusion protein. In addition, we found by histological assessment that treatment with p55 TNF receptor-IgG had resulted in a significant reduction in joint erosion (Table II) (Williams et al. 1995).

Studies to assess the effect of blocking IL-1 have also been carried out in CIA. Thus, IL-1ra and anti-IL-1β mAb both suppressed CIA when treatment was started before disease onset (Wooley et al. 1993b, Geiger et al. 1993). Furthermore, treatment after disease onset with polyclonal anti-IL-1α/β, or anti-IL-1β alone, profoundly suppressed both inflammation and cartilage destruction (Van den Berg et

TABLE II

Treatment	Dose	PIP joints with erosions
Saline	–	15/15 (100%)
Control mAb	300 μg	15/15 (100%)
Anti-TNF (TN3-19.12)	300 μg	9/15 (60%) P<0.05
Saline	–	11/12 (92%)
IgG control	100 μg	6/6 (100%)
TNF receptor-IgG (p55-sf2)	100 μg	6/12 (50%) P<0.05

Reductions in joint erosion following treatment of established CIA with anti-TNF mAb or p55 TNF receptor-IgG fusion protein. Both TNF-neutralizing agents were given three times over a 10 day period. At the end of the treatment period, sagittal sections of the PIP joints of the middle digits were studied in a blinded fashion for the presence or absence of erosions (defined as demarcated defects in cartilage or bone filled with inflammatory tissue). Based on original data published in Williams et al. (1992) and Williams et al. (1995).

such studies in terms of
GI anti-TNF monoclonal
y period, starting immedi-
ased a reduction in clinical
culating levels of anti-type
tological analysis showed
on in joint erosion relative
on of proximal-interphal-

nAbs containing epitopes
f a neutralizing antibody
n protein (of entirely hu-
f IgG and TNF receptor)
to mAbs, provided that
. In one study, a human
ice the clinical severity of
f arthritis (Wooley et al.
al efficacy of this form of
rec)-IgG fusion pro-
that treatment with p55
n in joint erosion (Table

been carried out in CIA.
hen treatment was started
993). Furthermore, treat-
or anti-IL-1β alone, pro-
ruction (Van den Berg et

IP joints with erosions
15/15 (100%)
15/15 (100%)
9/15 (60%) P<0.05
11/12 (92%)
6/6 (100%)
6/12 (50%) P<0.05

CIA with anti-TNF mAb or
gents were given three times
al sections of the PIP joints
sence or absence of erosions
nflammatory tissue). Based
ns et al. (1995).

al. 1994). It is clear, therefore, that IL-1 contributes significantly to the pathology of CIA. However, in the TNFα transgenic mouse, a disease model in which the arthritis is driven by TNFα, anti-IL-1 therapy also ameliorates disease (Kolias, personal communication). These data are consistent with our observations in RA, which demonstrate that TNFα regulates the production of IL-1, and is therefore a logical therapeutic target, although in CIA, unlike in RA, a TNFα-independent pathway of IL-1 production may exist in addition.

The studies described above indicate that inflammatory cytokine-targeted therapy is effective in reducing the severity of CIA, and our work on anti-TNF treatment also indicates that the duration of the therapeutic effect is dependent on the presence of excess anti-TNF mAb in the circulation. However, results in patients are different, with the clinical benefit outlasting the 'therapeutic levels' of antibody by several weeks (unpublished data).

Experiments on animal models have provided further data that may be of relevance for the treatment of human disease. Early studies, for example, demonstrated that anti-CD4 mAb could block the induction of CIA if injected close to the time of immunization (Ranges et al. 1985), and this kind of study was used as evidence to support the initiation of clinical trials of anti-CD4 in human RA (Herzog et al. 1987). In CIA, however, anti-CD4 treatment was found to be ineffective when given *after* collagen immunization (Brahn & Trentham 1984), a finding that was confirmed in a subsequent study which showed that anti-CD4 treatment alone was ineffective in established CIA (Hom et al. 1988). Anti-CD4 treatment in human RA has been shown in a number of open clinical trials to provide a degree of transient clinical benefit to some patients (Herzog et al. 1989), although many patients fail to respond to therapy in spite of severe peripheral T-cell depletion (Burmester et al. 1992, Choy et al. 1992). Furthermore, the results of at least one placebo-controlled trial of anti-CD4 therapy in RA clearly showed a lack of clinical benefit (van der Lubbe et al. 1994) and there is, therefore, a degree of concordance between human RA and murine CIA with respect to the relative lack of efficacy of anti-CD4 treatment in established disease.

Having established the distinction in the efficacy of anti-TNF and anti-CD4 therapy after disease onset we carried out a study to determine whether a combination of T cell-targeted therapy and TNF-targeted therapy would have a more significant impact on CIA than either treatment alone. Thus, DBA/1 mice with established CIA were given intraperitoneal injections of either a depleting anti-CD4 (YTS191.1.2/YTA3.1.2) alone, anti-TNF (TN3-19.12) alone or anti-CD4 plus anti-TNF. The results showed that anti-CD4 m/Ab acted synergistically with both a sub-optimal dose (50 μg) and an optimal dose (300 μg) of anti-TNF mAb (Williams et al. 1994). The beneficial therapeutic effects of anti-CD4 and anti-TNF were observed both clinically, in the suppression of paw-swelling and in the prevention of new limb involvement and histologically, in the protection against joint erosion of cartilage and bone. Mice treated with anti-TNF alone developed

a non-neutralizing antibody response to the anti-TNF mAb which was completely blocked by concurrent anti-CD4 treatment, a finding that may be important in terms of long-term treatment in man.

We were subsequently able to provide evidence of synergy between anti-CD4 and TNF receptor-IgG fusion protein (Williams et al. 1995). As in the previous experiment, it was found that concurrent anti-CD4 treatment almost completely blocked the anti-TNF receptor-IgG fusion protein response, which in this experiment resulted in increased circulating levels of the fusion protein. One of the possible mechanisms, therefore, to account for the synergy between anti-CD4 and TNF receptor-IgG is the prevention of a neutralizing antibody response leading to increased half-life of the fusion protein. However, other mechanisms of synergy also exist because synergy was observed between anti-CD4 and anti-TNF mAb in the absence of significant changes in levels of free anti-TNF in the serum (Williams et al. 1994).

In conclusion, these studies have shown that anti-TNF treatment leads to a degree of amelioration of CIA that is significantly increased when combined with anti-CD4. It is possible that combination therapy, targeted at the autoimmune response as well as the inflammatory cytokine network will provide the basis for the future treatment of RA.

EVIDENCE FOR ROLE ON TNFα FROM CLINICAL TRIALS

Having gathered *in vitro* and *in vivo* evidence of an important role for TNFα, the concept that neutralizing doses of monoclonal anti-TNFα antibody administered systemically might suppress RA was finally tested in patients by clinical trials. The aims of these trials was firstly to establish proof of principle, i.e., that it was possible to demonstrate clinical efficacy of an anti-TNFα chimeric monoclonal antibody (cA2) by employing validated clinical and laboratory parameters and showing a dose-response effect; secondly, to assess whether the magnitude and duration of benefit were sufficiently significant to herald a therapeutic advance; and thirdly, to investigate the safety and tolerability of the chimeric monoclonal antibody. To date, results of three clinical trials which have been completed on 93 patients have clearly demonstrated a substantial benefit in the short term in 80–90% of patients, following intravenous injection of cA2, with excellent tolerability and few side-effects.

The first study was designed primarily to test our hypothesis that TNF was of importance in the pathogenesis of RA. In this pilot study, we recruited 20 patients from our own hospital clinic and following referral from outside physicians. Each patient had refractory disease, in that they had a relatively long disease duration (median of 10.5 years), had either failed therapy or had reacted badly to several standard disease modifying antirheumatic drugs (DMARDS) and had continuing evidence of active synovitis. Although patients were permitted to continue on

stable doses of non-ste
steroids, any disease-mo
were withdrawn over a 4

The antibody used in t
USA) a chimerized (hum
for human TNFα, with n
Although this was the fir
matic disease and the do
from the CIA model as a
tered either 2 or 4 infusio
hours. The total dose deli

The patients were follo
measures of disease activ
by means of the Paulus c
six independent variable
mat of our data (Elliott
most encouraging. 19 of
patient dropping out at t
(bronchitis). Patients sh
the most rapid changes
and gradual but still mar
Other clinical measures
of response also showed
supported by significant

IL-6 and measure

Time (days after treatment)	(r
0 (entry)	*60
1	14
7	40
14	32
28	10
56	3

* Median (interquartile ra
** Not done.

*Paulus criteria: Significant
● at least 20% improvemen
ation of morning stiffnes
● at least 2 grade improver

stable doses of non-steroidal anti-inflammatory drugs and low dose corticosteroids, any disease-modifying drugs which the patients were taking at screening were withdrawn over a 4-week period, prior to entry.

The antibody used in the study and in subsequent trials was cA2 (Centocor, Pa, USA) a chimerized (human IgG_1/mouse F_v) monoclonal antibody with specificity for human TNFα, with maintenance of high affinity binding (Knight et al. 1993). Although this was the first occasion that such an antibody had been used in rheumatic disease and the dosage required was unclear, we had preliminary evidence from the CIA model as a guide to the dose range (Williams et al. 1992). We administered either 2 or 4 infusions of cA2 over a period of 2 weeks, each infusion lasting 2 hours. The total dose delivered in each treatment regime was the same (20mg/kg).

The patients were followed using a number of different clinical and laboratory measures of disease activity and the overall response to treatment was assessed by means of the Paulus criteria*, a composite disease activity index incorporating six independent variables (Paulus et al. 1990) modified to accommodate the format of our data (Elliott et al. 1993). The results from this small pilot study were most encouraging. 19 of the 20 completed all scheduled infusions, with the one patient dropping out at the 2-week infusion point because of a concomitant illness (bronchitis). Patients showed marked improvement in all clinical measures, with the most rapid changes seen in the duration of morning stiffness and pain score and gradual but still marked improvements in the swollen and tender joint counts. Other clinical measures including the grip strength and the patient's assessment of response also showed significant improvement. The clinical improvements were supported by significant falls in laboratory measures of disease activity including

TABLE III

IL-6 and measures of the acute phase response after treatment with cA2

Time (days after treatment)	Measure			
	IL-6 (pg/ml)	SAA (mg/ml)	CRP (mg/l)	ESR mm/hour
0 (entry)	*60 (42, 104)	245 (127, 513)	40 (28, 67)	55 (24, 77)
1	14 (1, 43)	190 (61, 415)	26 (18, 57)	**ND
7	40 (8, 60)	58 (26, 148)	5 (2, 13)	26 (19, 43)
14	32 (1, 51)	80 (38, 220)	6 (2, 29)	27 (15, 52)
28	10 (2, 48)	118 (44, 300)	11 (4, 32)	23 (16, 57)
56	3 (0, 14)	105 (54, 140)	6 (3, 25)	30 (21, 55)

* Median (interquartile range) of up to 20 patients per point.
** Not done.

*Paulus criteria: Significant improvement in at least 4 of 6 variables, defined as:
- at least 20% improvement in continuous variables (tender and swollen joint scores, duration of morning stiffness, ESR).
- at least 2 grade improvement in patient's and observer's assessment of disease severity.

the acute phase proteins, serum amyloid-A (SAA), C-reactive protein (CRP) and the erythrocyte sedimentation rate (ESR; Table III). The response in each of these measures was rapid, with maximal improvement from day 7 after treatment. Significant falls were also seen in serum levels of IL-6, an important cytokine involved in the hepatic acute phase response. For many patients, serum IL-6 levels had normalized by day 1 after treatment and remained low for the duration of the study (Table III and data not shown). Serial changes in serum IL-6, SAA and CRP in a patient with a good response to cA2 are shown in Fig. 3 and demonstrate normalization of IL-6 levels in advance of the acute phase proteins. The data are consistent with the hypothesis that TNF blockade leads to inhibition of IL-6 synthesis, with subsequent down-regulation of hepatic acute phase protein synthesis. Other laboratory measures which demonstrate a trend towards improvement included the white blood cell and platelet counts and the hemoglobin.

Although these results were obtained in an open label, uncontrolled trial, the consistency and extent of the clinical improvements and the parallel changes in laboratory measures of the acute phase response suggested that the responses

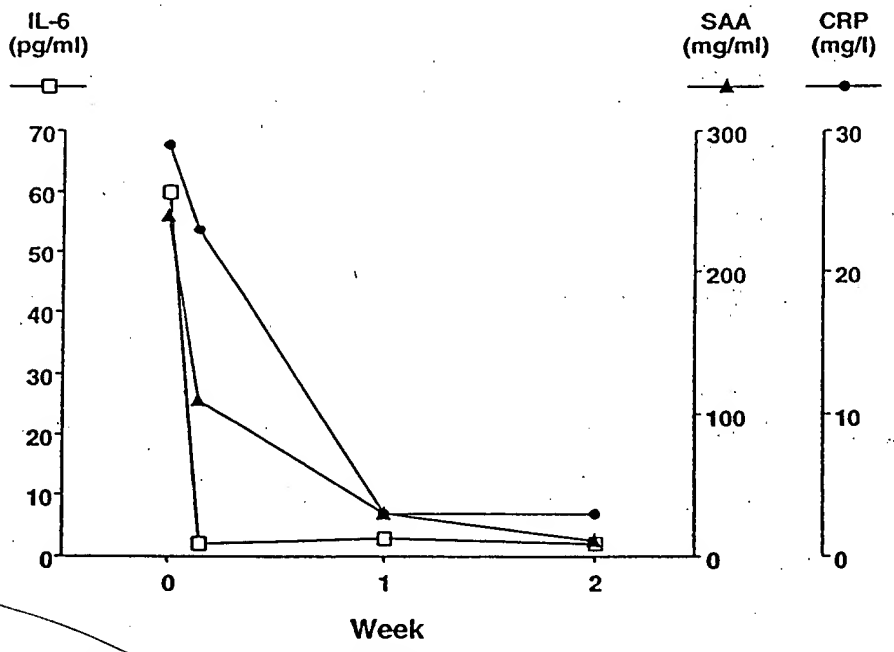


Figure 3. Changes in serum IL-6, SAA and CRP in a patient treated with cA2. The patient received a single infusion of 10 mg/kg cA2 at entry (week 0). IL-6 and SAA were measured by ELISA (Medgenix Diagnostics and Biosource International, respectively) and CRP were measured by rate nephelometry. Normal ranges: IL-6<10 pg/ml; SAA<10 mg/ml; CRP<10 mg/ml.

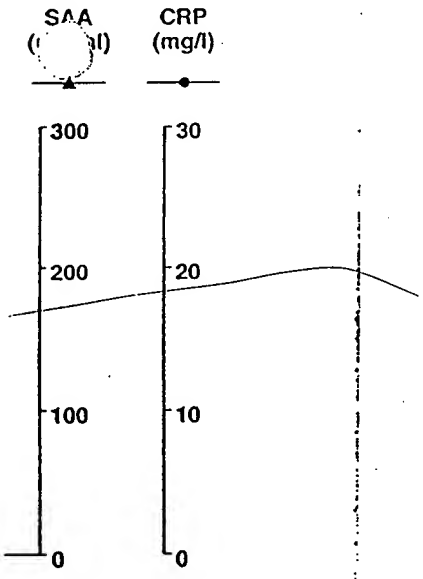
were likely to be du
importance of TNF
in this disease, we d
compared the use of
Many of the design
tory disease, the requ
for stable doses of o
study. Patients were
10 mg/kg) and were
The predetermine
week 4 of a response
there were substantia
the 24 placebo recipi
treated with high-do
intermediate, demon
of improvement was
end-point, then 58%
fits from treatment (
provements in indivi
lar changes were see
study. In particular,
ation of morning sti

20% Response

50% Response

Figure 4. Responses at w
cA2 or placebo. Patients
sponses were calculated
values represent significa

ve protein (CRP) and
response in each of
day 7 after treatment.
important cytokine
nts, serum IL-6 levels
v for the duration of
erum IL-6, SAA and
n Fig. 3 and demon-
phase proteins. The
leads to inhibition of
acute phase protein
a trend towards im-
and the hemoglobin.
ncontrolled trial, the
e parallel changes in
d that the responses



with cA2. The patient
ıd SAA were measured
ectively) and CRP were
A<10 mg/ml; CRP<10

were likely to be due to a true therapeutic effect. As the definitive test of the importance of TNF in the pathogenesis of RA and of the clinical efficacy of cA2 in this disease, we devised a multi-center, randomized, double-blind study which compared the use of cA2 to placebo (human serum albumin) (Elliott et al. 1994a). Many of the design features, inducing the requirement for longstanding, refractory disease, the requirement for withdrawal from DMARD therapy and the need for stable doses of other concomitant medications were maintained into the 2nd study. Patients were given a single infusion of placebo or cA2 (at a dose of 1 or 10 mg/kg) and were followed by blinded observers for response.

The predetermined, primary endpoint of the study was the achievement at week 4 of a response according to the Paulus (20%) criterion. Using this measure there were substantial and clear differences between the 3 groups, with only 2 of the 24 placebo recipients (8%) responding compared with 19 of 24 patients (79%) treated with high-dose cA2. The responses of the low-dose cA2 recipients were intermediate, demonstrating a dose-response relationship (Fig. 4). If the threshold of improvement was raised to a 50% improvement in Paulus index at a 4-week end-point, then 58% of patients treated with the higher dose of cA2 gained benefits from treatment (Fig. 4). Although considered supportive in nature, the improvements in individual disease activity measures were also of interest and similar changes were seen in the cA2 treated groups to those seen in the open label study. In particular, the tender and swollen joint counts, the pain score, the duration of morning stiffness, the fatigue score, the grip strength and the patients'

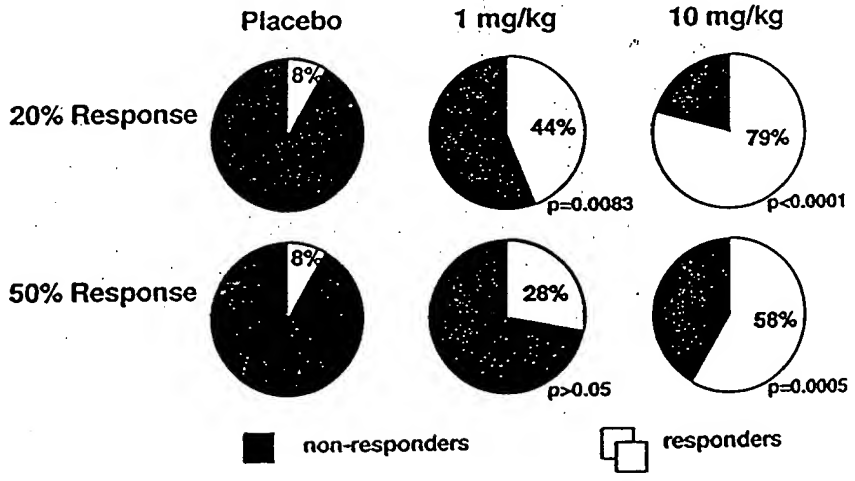


Figure 4. Responses at week 4 in patients treated with high (10 mg/kg) or low (1 mg/kg) dose cA2 or placebo. Patients received a single infusion of cA2 or placebo at entry (week 0). Responses were calculated according to Paulus 20% or 50% criteria, as described in the text. p values represent significance of changes compared with placebo by Fisher's exact test.

and observers' assessments of disease severity all showed highly significant improvements. These changes were matched by improvements in the ESR and CRP, particularly in the high-dose cA2 group. As in the open label study, the infusions were well tolerated and though a variety of adverse events were seen, the overall safety profile of cA2 was good, and the adverse events were not greater at high doses of cA2 than placebo.

One element of particular interest in this study was the improvement in the hemoglobin concentration in patients treated with high-dose cA2, compared with a fall in placebo-treated patients. Although these changes were small in magnitude, they occurred over a period of only 4 weeks in the setting of significant venesection for safety monitoring, and the difference between the changes in placebo and high-dose cA2 groups was highly significant. The anemia of chronic disease seen in RA is multifactorial in origin but recent evidence suggests that TNFα has a direct effect in the bone marrow in suppressing erythropoiesis (Roodman 1987, Johnson et al. 1989). While the improvements in hemoglobin in cA2 treated patients might be simply a reflection of improvement of overall disease state, a more interesting hypothesis is that cA2 is directly interfering with TNFα mediated suppression of erythropoiesis in the bone marrow.

The results of this study confirm that cA2 is a highly effective agent in the short-term suppression of RA and strongly support the hypothesis that TNF is a significant contributor to the inflammtory state in this disease. The findings do not however directly address the question of the relative importance of TNFα and IL-1 in disease pathogenesis. No full-length reports are yet available describing the use of specific IL-1 blockers in RA, but preliminary data presented in abstract form suggest some therapeutic efficacy for both IL-1ra (Lebsack et al. 1993) and a soluble receptor for IL-1 (Drevlow et al. 1993).

The data presented so far suggests that cA2 may find a role as a remission inducing agent in RA, or in the control of acute disease flares. A more ambitious goal for a new therapeutic agent, however, would be long-term disease suppression and in particular the achievement of disease modification. Although many of the currently available drugs in the treatment of rheumatic disease are termed 'disease modifying', the evidence that they do in fact alter outcomes is at best arguable (Brooks 1993). TNFα has major effects on cartilage metabolism, including the inhibition of collagen synthesis and the stimulation of collagenase production by fibroblasts and synovial cells, together with both direct and indirect effects on proteoglycan metabolism (reviewed by Vassalli 1992). It might be expected, therefore, that long-term TNF blockade would favorably influence cartilage metabolism and reduce tissue damage.

Experience with cA2 suggests that, although it is a reliable disease suppressing agent, the responses are transient, with patients showing disease relapse after a period of months after the antibody disappears from the circulation. In our first experience in the repeated use of cA2, we administered cycles of therapy to a small

number of patients ori
pleted between 2 and
upon evidence of disea
(Elliott et al. 1994b), v
maintenance of respons
sponse duration. Of p
genicity, since the murir
murine responses upon
anti-murine response in
patients re-treated with

The clinical significa
are as yet unclear, but
long-term treatment ei
blocking cA2 binding si
sent the major therapeu
engineered thereapeutic
tially immunogenic (Is
adopted to circumvent
bodies to CD4 to block
administered anti-TNF
mal models.

Preliminary reports
made recently. In one,
to patients with active
tion study (Rankin et
improvements were see
cluding the tender join
study employed a fusio
tor coupled to a huma
ranging study incorpor
cutaneous administrati
cant improvements we
joint counts, the patie
morning stiffness, toget
and further validate th
of trials in progress w
summarized in Table I

MECHANISM O

The 'pleiotropy' of TNI
types, driving multiple

number of patients originally enrolled in the open label trial. Seven patients completed between 2 and 4 complete treatment cycles, with each cycle administered upon evidence of disease relapse. The findings have been reported in full recently (Elliott et al. 1994b), with repeated responses after each cycle administered, and maintenance of response magnitude, but a trend (non-significant) to shortening response duration. Of particular interest in this study was the tissue of immunogenicity, since the murine variable region of cA2 might be expected to stimulate anti-murine responses upon repeated dosing. Although only 1 patient had developed an anti-murine response in the original open label trial (unpublished data), 50% of the patients re-treated with cA2 developed such responses, mostly at a low titer.

The clinical significance of these immunological responses to injected antibody are as yet unclear, but it is possible that they could limit therapeutic efficacy with long-term treatment either through accelerated clearance of injected cA2, or by blocking cA2 binding sites to TNFα. Overcoming these responses is likely to represent the major therapeutic challenge for the repeated use of cA2, or indeed any other engineered thereapeutic antibodies, since even 'humanized' antibodies are potentially immunogenic (Isaacs et al. 1992). While a number of approaches could be adopted to circumvent the development of such responses, administration of antibodies to CD4 to block the development of antiglobulin responses to concurrently administered anti-TNF antibody has been discussed in the previous section on animal models.

Preliminary reports from 2 other studies targeting TNFα in RA have been made recently. In one, a humanized murine antibody (CDP571) was administered to patients with active rheumatoid arthritis in a placebo controlled, dose escalation study (Rankin et al. 1994). The antibody was well tolerated and significant improvements were seen in a number of individual disease activity measures, including the tender joint count, the pain score and the ESR. A second reported study employed a fusion protein, comprising recombinant human p75 TNF receptor coupled to a human IgG1 Fc framework (Moreland et al. 1994). This dose-ranging study incorporated intravenous loading at entry, with twice-weekly subcutaneous administration of the fusion protein for maintenance therapy. Significant improvements were seen compared to baseline for the tender and swollen joint counts, the patient and physician global assessments and the duration of morning stiffness, together with the ESR. These data lend support to our findings and further validate the clinical efficacy of TNFα blockade. The current status of trials in progress with anti-cytokines mostly published in abstract form, is summarized in Table IV.

MECHANISM OF ACTION OF ANTI-TNFα ANTIBODY THERAPY

The 'pleiotropy' of TNF action, jargon for its multitude of effects on various cell types, driving multiple biological processes, is the likely reason for the marked

TABLE IV
Anti-cytokines in clinical trials in RA

Target	Therapy	Results
TNFα	● Monoclonal anti-TNFα antibody:	
	– Chimeric (cA2) (Centocor)+	*RCT: beneficial
	– humanised (CDP571) (CellTech)	Dose escalation: beneficial
	● Soluble TNF-R-Ig fusion proteins:	
	– TNFR:Fc (p55) (Roche)	In trials: beneficial
	– TNFR:Fc (p75) (Immunex)	Dose ranging: beneficial
IL-1	● IL-1RA (Synergen)	Dose ranging: beneficial
	● Soluble IL-1R (Immunex)	RCT: trend to beneficial
IL-6	● Monoclonal anti-IL-6 antibody	
	– murine (BE-8)	Open label: beneficial

*RCT=randomized placebo-controlled trial.
+=published as full papers, rest are published in abstract form.

effects of anti-TNF therapy *in vivo*. However, it remains to be established which of these many effects of TNF, inhibited by anti-TNF, are of central importance in the therapeutic effect. Our current working hypothesis, based on the results so far that the cA2 anitbody has two major effects. It interrupts the cytokine cascade *in vivo*, just as it does in the *in vitro* rheumatoid synovial cultures. The best clinical evidence for interruption of the cytokine cascade *in vivo* is the rapid and marked reduction in the CRP, the rise of which in RA reflects the cytokine activation of the hepatocytes, probably chiefly by IL-6, but possibly involving any of the cytokines whose signals are mediated by gp130 (e.g. LIF, IL-11). Supporting evidence for widespread cytokine blockade has been obtained by the fall in serum IL-6 levels by ELISA (Fig. 3). Cytokine blockade and diminution of events downstream is probably of major importance in the rapidity of onset of clinical benefit, and in the reduction in local inflammatory features, such as morning stiffness, pain and joint swelling.

However, an important feature of cA2 therapy is the duration of clinical benefit, much beyond the fall of serum levels of anti-TNF to levels not capable of neutralizing TNFα bioactivity within the RA joints. A number of lines of evidence have converged to suggest the hypothesis that cA2 has a major effect on the recruitment and trafficking of blood cells to the joint. Endothelium in RA synovium has been reported to express increased levels of adhesion molecules for leukocytes, such as E-selectin, VCAM-1 and ICAM-1 (Koch et al. 1991, Fischer et al. 1993, Johnson et al. 1993, Cronstein 1994, Szekanecz et al. 1994). Since blood cells, neutrophils, but especially macrophages and T cells, are needed for maintaining the disease process and do not proliferate much locally, interruption of the influx of cells may be of major significance in the duration and depth of

Figure 5. Reduction of anti-TNFα monoclona 14 days after cA2 infus the degree of inflamm by scoring the thickne aggregates (Firestein e the two sections of eac

benefit. Moreover, it destructive process it reducing the clinical existing drugs.

The clues we have synovial histology, w also been studied, a vation, soluble E-sele

Synovial biopsies t patients in our first were taken from the s from each block of bi lining layer, cellular i parameters were used brane. We found the 1. In the 3 patients w from a mean value of

A

sults

CT: beneficial
se escalation: beneficial

trials: beneficial
se ranging: beneficial

se ranging: beneficial
T: trend to beneficial

en label: beneficial

n.

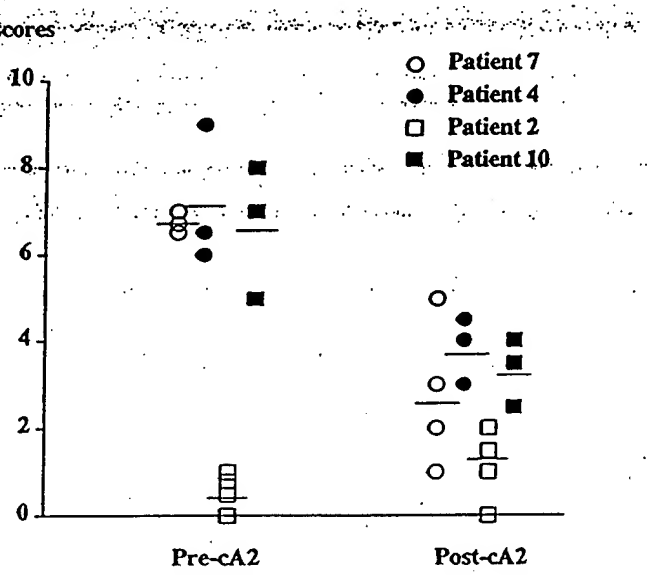


Figure 5. Reduction of inflammation in the synovial membrane in RA patients treated with anti-TNFα monoclonal antibody (cA2). Multiple synovial biopsies were taken before and 14 days after cA2 infusion from 4 patients in the 'open-label' clinical trial and analyzed for the degree of inflammation. Two distant sections from each biopsied sample were graded by scoring the thickness of the lining layer, cellular infiltration and the number of lymphoid aggregates (Firestein et al. 1991). Each point in the figure represents the average value of the two sections of each biopsy.

s t established which
re of central importance
is, based on the results so
upts the cytokine cascade
cultures. The best clinical
is the rapid and marked
he cytokine activation of
nvolving any of the cyto-
-11). Supporting evidence
by the fall in serum IL-6
inution of events down-
f onset of clinical benefit,
ch as morning stiffness,

duration of clinical bene-
to levels not capable of
number of lines of evi-
A2 has a major effect on
int. Endothelium in RA
f adhesion molecules for
Koch et al. 1991, Fischer
anecz et al. 1994). Since
d T cells, are needed for
much locally, interruption
e duration and depth of

benefit. Moreover, it suggests that blocking TNF long-term could interrupt the destructive process itself, which needs cell influx to sustain it, rather than merely reducing the clinical features such as pain and swelling more effectively than existing drugs.

The clues we have obtained to support the role of trafficking have come from synovial histology, where the adhesion molecules on synovial endothelium have also been studied, and from analysis of the serum marker of endothelial activation, soluble E-selectin.

Synovial biopsies before and 14 days after cA2 infusion were available from 4 patients in our first 'open-label' clinical trial. In each patient, at least 3 biopsies were taken from the same knee joint before and after therapy. Two distant sections from each block of biopsied tissue were stained and examined for thickness of the lining layer, cellular infiltration and the number of lymphoid aggregates. These parameters were used to score the degree of inflammation of the synovial membrane. We found the score to be high in 3 patients and only minimally raised in 1. In the 3 patients with the highest inflammatory score, there was a reduction from a mean value of 6.8 to 3.5 following cA2 therapy (Fig. 5). Other histological

features were assessed. The synovial lining layer is relatively uniform in individual joints, in contrast to the sublining layer which shows regional variability. The lining layer decreased from 6–8 cells thick to 3–4 cells thick following therapy. Lymphoid aggregates are patchy in distribution; nevertheless these were scored and showed a reduction from 5 to 3.5 per low-power field. Since neovascularization is a prominent feature of the RA synovium, the number of blood vessels (defined by staining with antibody to von Willebrand Factor and a specific anti-endothelial antibody EN4) (Ruiter et al. 1989) was compared. There was no significant change in the number of vessels in pre- and post-treatment specimens.

Since trafficking of leukocytes to the joint is an important process in the pathogenesis of rheumatoid arthritis and augmented expression of endothelial adhesion molecules is involved in this process, we monitored the expression of ICAM-1, VCAM-1 and E-selectin on vascular endothelium in the biopsies. ICAM-1 and VCAM-1 were detected on most vascular endothelial cells, as well as many of the lining layer cells. This is expected, based on the expression of these molecules on antigen-presenting cells as well as endothelial cells. There was a tendency for VCAM-1 staining on endothelial cells, but not on lining cells, to be reduced post-treatment, but no changes in ICAM-1 expression on endothelium were noted. Antibody to E-selectin exclusively stained 3–28% of vascular endothelial cells on capillaries and venules. The expression of E-selectin was significantly reduced in the post-treatment samples (Fig. 6).

Recently it has become apparent that many adhesion molecules, like cytokine receptors, also exist in the serum in a soluble form, to complement the cell surface form. Their function in the serum is not clearly documented, and unlike sTNF-R, which was discovered as a urinary TNF inhibitor, it is not yet known whether they are competitive inhibitors. However, it is known that these molecules are derived from proteolytic cleavage of the surface form, as no alternatively spliced mRNA has been found, at least for ICAM-1 and E-selectin (Gearing & Newman 1993). Release of soluble ICAM-1 and E-selectin has been found to correlate with expression of these molecules on the surface of cytokine-activated cultured endothelial cells (Leeuwenberg et al. 1992, Pigott et al. 1992). Elevated serum levels of ICAM-1, VCAM-1 and E-selectin have been observed in RA (Cush et al. 1993, Koch et al. 1993, Wellicome et al. 1993), although only ICAM-1 and VCAM-1 appear to correlate with disease severity (ESR) (Aoki et al. 1993, Mason et al. 1993).

In order to further assess the significance of the reduction in E-selectin observed on synovial blood vessels, levels of soluble E-selectin were assayed in serial serum samples, and were found to be diminished by cA2 therapy in 19/20 patients. The earliest observed decrease was at day 7, although serum levels appeared to be attenuated even at 4 and 6 weeks (Fig. 7). In some patients levels returned to baseline by day 56 (in 5/20 levels were rising by weeks 6 to 8); however, in most patients levels were still below pre-treatment levels at that time. The initial levels

E-selectin-st
blood vessel
3(
2(
2(
1(
1(
5
(

Figure 6. Decrease of E
in RA patients treated
(as stated in Fig. 1) were
(BBA1). The expression
samples in the same 3 p

in the 20 patients var
ml). In 1 patient, in
served, the initial lev

Levels of soluble I
an apparent decreas
creased from 351.7 r
difference was not s
tested. Four weeks p
to pre-infusion value
soluble VCAM-1. Th
uble VCAM-1 at any

Rheumatoid arthritis
therapies are of limit
initial approcah to u

uniform in individual
ional variability. The
ick following therapy.
ess these were scored
. Since neovasculariz-
nber of blood vessels
or and a specific anti-
ed. There was no sig-
eatment specimens.
process in the patho-
endothelial adhesion
pression of ICAM-1,
iopsies. ICAM-1 and
is well as many of the
of these molecules on
was a tendency for
s, to be reduced post-
othelium were noted.
r er helial cells on
gnifi ly reduced in

lecules, like cytokine
ement the cell surface
d, and unlike sTNF-
t yet known whether
these molecules are
alternatively spliced
(Gearing & Newman
n found to correlate
ne-activated cultured
92). Elevated serum
rved in RA (Cush et
h only ICAM-1 and
oki et al. 1993, Ma-

on in E-selectin ob-
were assayed in serial
apy in 19/20 patients.
n levels appeared to
its levels returned to
8); however, in most
ne. The initial levels

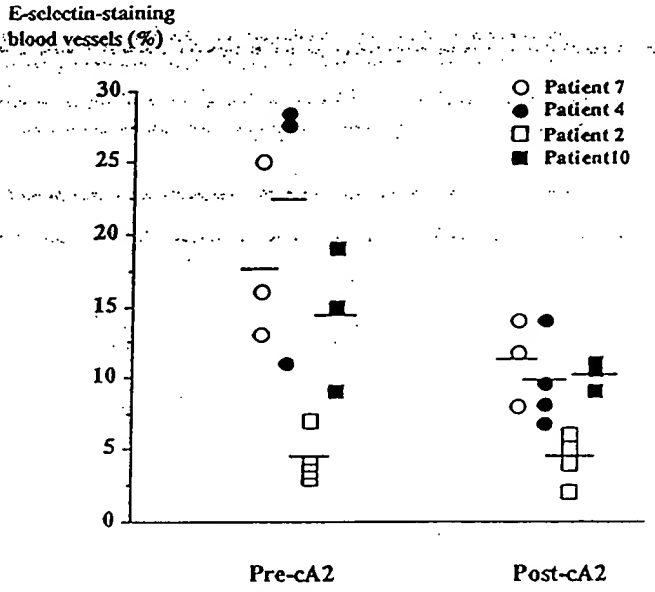


Figure 6. Decrease of E-selectin expression on endothelial cells in the synovial membrane in RA patients treated with anti-TNFα monoclonal antibody (cA2). The synovial biopsies (as stated in Fig. 1) were stained with a mouse monoclonal anti-human E-selectin antibody (BBA1). The expression of E-selectin on endothelial cells was reduced in the post-treatment samples in the same 3 patients that showed reduced inflammatory scores.

in the 20 patients varied considerably from 41–224 ng/ml (mean normal 47.6 ng/ml). In 1 patient, in whom no diminution of soluble E-selectin levels was observed, the initial levels were in the normal range (<50ng/ml).

Levels of soluble ICAM-1 were assayed in only 3 patients, and also exhibited an apparent decrease following cA2 therapy. Mean serum ICAM-1 levels decreased from 351.7 ng/ml (pre-infusion) to 262.3 ng/ml by day 7, although this difference was not statistically significant due to the low number of patients tested. Four weeks post-infusion the levels of ICAM-1 appeared to be returning to pre-infusion values. Finally, sera from a total of 10 patients were assayed for soluble VCAM-1. There was no significant difference detectable in levels of soluble VCAM-1 at any time after cA2 infusion (Fig. 7).

SUMMARY

Rheumatoid arthritis is a common cause of chronic disability for which current therapies are of limited value in controlling the disease process and outcome. Our initial approcah to understanding the pathogenesis of RA and defining a novel

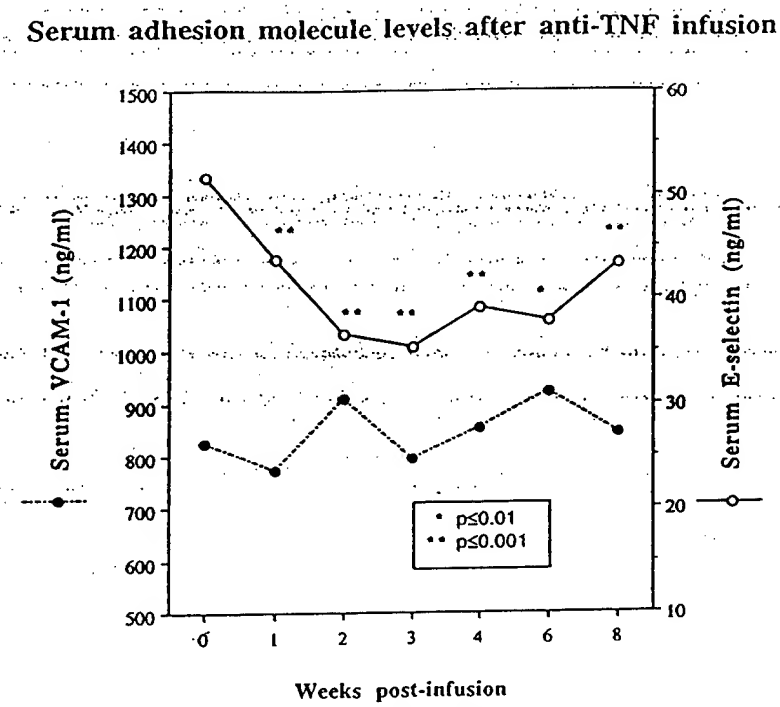


Figure 7. Serum adhesion molecules after anti-TNF infusion. Circulating levels of serum E-selectin (-o-) VCAM −1 (-●-) were measured by ELISA (British Biotechnology Products Ltd, UK) after infusion of cA2. Values are means of 20 and 10 patients respectively. Significant differences versus pre-infusion values were determined by Wilcoxon signed rank test (*) $p<0.01$, (**) $p<0.001$.

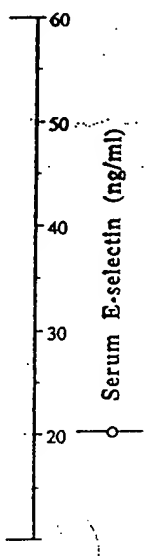
therapeutic target was to investigate the role of cytokines by blocking their action with antibodies on cultured synovial-derived mononuclear cells *in vitro*. These investigations suggested that neutralization of TNFα with antibodies significantly inhibited the generation of other pro-inflammatory cytokines also over-produced, such as, IL-1, GM-CSF, IL-6 and IL-8. The implication that blockade of a single cytokine, TNFα might have far-reaching effects on multiple cytokines and thereby exert significant anti-inflammatory and protective effects on cartilage and bone of joints, was tested in arthritic DBA/1 mice immunized with collagen II. Impressive amelioration of joint swelling and joint erosions in this model encouraged clinical trials with a monoclonal anti-TNFα antibody. The cA2 chimeric anti-TNFα high-affinity antibody was initially tested in an open-label study at a dose of 20 mg/kg on 20 patients, with substantial and universal benefit. Subsequently, a randomized placebo-controlled double-blind trial was performed on 73 patients comparing a

single intravenous i
doses of cA2. Using
8% of patients recei
cA/2 and 79% recei
administered to 7 pa
nitude with each cyc
cated in the pathoge
anti-TNFα antibodi
term control of RA.
idiotypic responses i
ation of response. I
and anti-CD4 is obs
indicating one way i

The Kennedy Institu
funded research inst
for preparation of t

Aoki, S., Imai. K. & Y
antigen in patient
Bevilacqua, M. P. (19
Immunology, eds.
Inc. California, U
Brahn, E., Peacock, D.
factor alpha (TNF
Brahn, E. & Trentham
rats: evidence that
Brennan, F. M., Chant
effect of TNFα a
arthritis. *Lancet* 2
Brennan, F. M., Chant
Detection of trans
lack of effect on
Immunol. 81, 278.
Brennan, F. M., Zacha
Matsushima, K. &
in synovial fluids
mRNA by isolate
Brennan, F. M., Field.
expression in rheu
Brooks, P. M. (1993) C
Buchan, G., Barrett, K

NF infusion



ting levels of serum E-
iotechnology Products
its respectively. Signifi-
oxon signed rank test

locking their action
cells *in vitro*. These
tibodies significantly
also over-produced,
blockade of a single
rtokines and thereby
artilage and bone of
llagen II. Impressive
encouraged clinical
ric anti-TNFα high-
a dose of 20 mg/kg
iently, a randomized
atients comparing a

single intravenous injection of placebo (0.1% human serum albumin) with two doses of cA2. Using a composite disease activity index, at 4 weeks post infusion, 8% of patients receiving placebo improved compared with 44% receiving 1mg/kg cA/2 and 79% receiving 10 mg/kg. Between 2 to 4 repeated cycles of cA2 were administered to 7 patients and all patients showed improvement of a similar magnitude with each cycle. These data support our proposition that TNFα is implicated in the pathogenesis of RA, and is thus a key therapeutic target. Monoclonal anti-TNFα antibodies control disease flares and are candidate agents for longer-term control of RA, although repeated therapy with cA2 is associated with anti-idiotypic responses in 50% of patients and a trend toward shortening of the duration of response. In the DBA/1 arthritic mice, synergy of action of anti-TNF and anti-CD4 is observed together with suppression of an anti-globulin response, indicating one way in which benefit might be augmented in the future.

ACKNOWLEDGMENTS

The Kennedy Institute of Rheumatology is an Arthritis and Rheumatism Council funded research institute. We thank Ms. Jennifer Linfield and Ms. Lindsay Roffe for preparation of the manuscript.

REFERENCES

Aoki, S., Imai, K. & Yachi, A. (1993) Soluble intercellular adhesion molecule-1 (ICAM-1) antigen in patients with rheumatoid arthritis. *Scand. J. Immunol.* 38, 485.

Bevilacqua, M. P. (1993) Endothelial-leukocyte adhesion molecules. In: *Annual Review of Immunology*, eds. Paul, W. E., Fathman, C. G. & Metzger, H., p. 767. Annual Reviews Inc. California, USA.

Brahn, E., Peacock, D. J., Banquerigo, M. L. & Liu, D. Y. (1992) Effects of tumor necrosis factor alpha (TNF-α) on collagen arthritis. *Lymphokine & Cytokine Res.* 11, 253.

Brahn, E. & Trentham, D. E. (1984) Effect of antithymocyte serum on collagen arthritis in rats: evidence that T cells are involved in its pathogenesis. *Cell. Immunol.* 86, 421.

Brennan, F. M., Chantry, D., Jackson, A., Maini, R. N. & Feldmann, M. (1989) Inhibitory effect of TNFα antibodies on synovial cell interleukin-1 production in rheumatoid arthritis. *Lancet* 2, 244.

Brennan, F. M., Chantry, D., Turner, M., Foxwell, B., Maini, R. N. & Feldmann, M. (1990a) Detection of transforming growth factor-beta in rheumatoid arthritis synovial tissue: lack of effect on spontaneous cytokine production in joint cell cultures. *Clin. Exp. Immunol.* 81, 278.

Brennan, F. M., Zachariae, C. O., Chantry, D., Larsen, C. G., Turner, M., Maini, R. N., Matsushima, K. & Feldman, M. (1990b) Detection of interleukin 8 biological activity in synovial fluids from patients with rheumatoid arthritis and production of IL-8 mRNA by isolated synovial cells. *Eur. J. Immunol.* 20, 2141.

Brennan, F. M., Field, M., Chu, C. Q., Maini, R. N. & Feldmann, M. (1991) Cytokine expression in rheumatoid arthritis. *Br. J. Rheumatol.* 1, 76.

Brooks, P. M. (1993) Clinical management of rheumatoid arthritis. *Lancet* 341, 286.

Buchan, G., Barrett, K., Fujita, T., Taniguchi, T., Maini, R. N. & Feldman, M. (1988a)

Detection of activated T cell products in the rheumatoid joint using cDNA probes to interleukin 2, IL-2 receptor, and interferon γ. *Clin. Exp. Immunol.* 71, 295.

Buchan, G., Barrett, K., Turner, M., Chantry, D., Maini, R. N. & Feldmann, M. (1988b) Interleukin-1 and tumour necrosis factor mRNA expression in rheumatoid arthritis: prolonged production of IL-1 alpha. *Clin. Exp. Immunol.* 73, 449.

Burmester, G. R., Horneff, G. & Emmrich, F. (1992) Intervention with immunomodulatory agents: monoclonal antibody therapy. *Baillieres Clin. Rheumatol.* 6, 415.

Butler, D., Maini, R. N., Feldmann, M. & Brennan, F. M. Blockade of TNFα with chimeric anti TNFα monoclonal antibody, cA2 reduces (IL-6 and IL-8) release in RA NMC cultures: A comparison with IL-1ra. (manuscript submitted).

Chen, Y., Kuchroo, V. K., Inoba, J-I., Hafler, D. A. & Weiner, H. L. (1994) Regulatory T cell clones induced by oral tolerance: suppression of autoimmune encephalomyelytis. *Science* 265, 1237.

Chikanza, I. C., Petrou, P. Kingsley, G., Chrousos, G. & Panayi, G. S. (1992) Defective hypothalamic response to immune and inflammatory stimuli in patients with rheumatoid arthritis. *Arthritis Rheum.* 35, 1281.

Choy, E. H., Chikanza, I. C., Kingsley, G., Corrigall, V. & Panayi, G. S. (1992) Treatment of rheumatoid arthritis with single dose or weekly pulses of chimaeric anti-CD4 monoclonal antibody. *Scand. J. Immunol.* 36, 291.

Chu, C. Q., Field, M., Feldmann, M. & Maini, R. N. (1991) Localization of tumor necrosis factor alpha in synovial tissues and at the cartilage-pannus junction in patient with rheumatoid arthritis. *Arthritis Rheum.* 34, 1125.

Chu, C. Q., Field, M., Allard, S., Abney, E., Feldmann, M. & Maini, R. N. (1992) Detection of cytokines at the cartilage/pannus junction in patients with rheumatoid arthritis: implications for the role of cytokines in cartilage destruction and repair. *Br. J. Rheumatol.* 31, 653.

Cohen, S. B. A., Katsikis, P., Chu, C. Q., Thomssen, H., Webb, L. M. C., Maini, R. N., Londei, M. & Feldmann, M. (1995) Activated T cell population within the rheumatoid arthritis membrane has a skewed cytokine profile. *Arthritis Rheum* (in press).

Cope, A., Aderka, D., Doherty, M., Engelmann, H., Gibbons, D., Jones, A. C., Brennan, F. M., Maini, R. N., Wallach, D. & Feldman, M. (1992) increased levels of soluble tumour necrosis factor (TNF) receptors in the sera and synovial fluids of patients with rheumatic diseases. *Arthritis Rheum.* 35, 1160.

Cooper, W. O., Fava, R. A., Gates, C. A., Cremer, M. A. & Townes, A. S. (1992) Acceleration of onset of collagen-induced arthritis by intra-articular injection of tumour necrosis factor or transforming growth factor-beta. *Clin. Exp. Immunol.* 89, 244.

Cronstein, B. N. (1994) Adhesion molecules in the pathogenesis of rheumatoid arthritis. *Curr. Opin. Rheumatol.* 6, 300.

Cush, J. J., Rothlein, R., Lindsley, H. B., Mainolfi, E. A. & Lipsky, P. E. (1993) Increased levels of circulating intercellular adhesion molecule 1 in the sera of patients with rheumatoid arthritis. *Arthritis Rheum.* 36, 1098.

Dayer, J-M., Beutler, B. & Cerami, A. (1985) Cachectin/tumour necrosis factor stimulates collagenase and prostaglandin E2 production by human synovial cells and dermal fibroblasts. *J. Exp. Med.* 162, 2163.

Deleuran, B. W., Chu, C. Q, Field, M., Brennan, F.M., Katsikis, P., Feldmann, M. & Maini, R. N. (1992a) Localisation of the Interleukin-1α, type I Interleukin-1 receptor and Interleukin-1 receptor antagonist protein in the synovial membrane and cartilage/pannus junction in rheumatoid arthritis. *Br. J. Rheumatol.* 31, 801.

Deleuran, B. W., Chu, C. Q., Field, M., Brennan, F. M., Mitchell, T., Feldmann, M. & Maini R. N. (1992b) Localization of tumor necrosis factor receptors in the synovial tissue and cartilage-pannus junction in patients with rheumatoid arthritis. Implications for local actions of tumor necrosis factor α. *Arthritis Rheum.* 35, 1170.

Drevlow, B., Capezi
study of recom
articularly in ac

Elliott, M. J. & Mai
arthritis. *Int. A*

Elliott, M. J., Maini,
F. M., Walker, J
arthritis with c
Rheum. 36, 168

Elliott, M. J., Maini
B., Breedveld, F
double-blind co
α (cA2) versus

Elliott, M. J., Maini
J. N. (1994b) Re
(cA2) in patient

Erhardt, C. C., Mun
poor life progn
Rheum. Dis. 48,

Evavold, B. D., Sloa
functions stimul

Feldmann, M., Bren
disease. In: *Clin*
apy, eds. Oppen
Press.

Firestein, G. S. & Z
synovitis? *Arthr*

Fischer, C., Thiele,
inflamed synovi
Scand. J. Immu

Gearing, A. J. H. &
nology Today 14

Geiger, T., Towbin, H
K. (1993) Neutr
alleviates collag
phase response.

Gowen, M., Wood,
interleukin-1 lik

Gregersen, P. K., Sil
approach to un
thritis. *Arthritis*

Haworth, C., Brenn
(1991) Expressic
arthritis: regulat

Herzog, C., Walker
Knapp, W., Rie
2, 1461.

Herzog, C., Walker,
arthritis. *Concep*

Hirano, T., Matsuda
M., Maini, R.
stimulatory fact

Drevlow, B., Capezio, J., Lovis, R., Jacobs, C., Laday, A. & Pope, R. M. (1993) Phase I study of recombinant human interleukin-1 receptor (RHUIL-1R) administered intra-articularly in active rheumatoid arthritis. *Arthritis Rheum.* 36, (supp), S39.

Elliott, M. J. & Maini, R. N. (1994) New directions for biological therapy in rheumatoid arthritis. *Int. Arch. Allergy. Immunol.* 104, 112.

Elliott, M. J., Maini, R. N., Feldmann, M., Long-Fox, A., Charles, P., Katsikis, P., Brennan, F. M., Walker, J., Bijl, H., Ghrayeb, J. & Woody, J. N. (1993) Treatment of rheumatoid arthritis with chimeric monoclonal antibodies to tumor necrosis factor α. *Arthritis Rheum.* 36, 1681.

Elliott, M. J., Maini, R. N., Feldmann, M., Kalden, J. R., Antoni, C., Smolen, J. S., Leeb, B., Breedveld, F. C., Macfarlane, J. D., Bijl, H. & Woody, J. N. (1994a) Randomised double-blind comparison of chimeric monoclonal antibody to tumour necrosis factor α (cA2) versus placebo in rheumatoid arthritis. *Lancet* 344, 1105.

Elliott, M. J., Maini, R. N., Feldmann, M., Long-Fox, A., Charles, P., Bijl, H. & Woody, J. N. (1994b) Repeated therapy with monoclonal antibody to tumour necrosis factor α (cA2) in patients with rheumatoid arthritis. *Lancet* 344, 1125.

Erhardt, C. C., Mumford, P. A., Venables, P. J. & Maini, R. N. (1989) Factors predicting a poor life prognosis in rheumatoid arthritis: an eight year prospective study. *Ann. Rheum. Dis.* 48, 7.

Evavold, B. D., Sloan-Lancaster, J. & Allen, P. M. (1993) Tickling the TCR: selective T-cell functions stimulated by altered peptide ligands. *Immunology Today* 14, 602.

Feldmann, M., Brennan, F. M. & Maini, R. N. (1993) Role of cytokines in autoimmune disease. In: *Clinical applications of cytokines: Role in pathogenesis, diagnosis and therapy*, eds. Oppenheim, J. J., Rossio, J. L., & Gearing, A. J. H., p. 109. Oxford University Press.

Firestein, G. S. & Zvaifler, N. J. (1990) How important are T cells in chronic rheumatoid synovitis? *Arthritis Rheum* 33, 768.

Fischer, C., Thiele, H. G. & Hamann, A. (1993) Lymphocyte-endothelial interactions in inflamed synovia: involvement of several adhesion molecules and integrin epitopes. *Scand. J. Immunol.* 38, 158.

Gearing, A. J. H. & Newman, W. (1993) Circulating adhesion molecules in disease. *Immunology Today* 14, 506.

Geiger, T., Towbin, H., Cosenti-Vargas, A., Zingel, O., Arnold, J., Rordorf, C. & Vosbeck, K. (1993) Neutralization of interleukin-1β activity in vivo with a monoclonal antibody alleviates collagen-induced arthritis in DBA/1 mice and prevents the associated acute-phase response. *Clin. Exp. Rheumatol.* 11, 515.

Gowen, M., Wood, D. D., Ihrie, E. J., Mcguire, M. K. B. & Russell, R. G. (1983) An interleukin-1 like factor stimulates bone resorption *in vitro*. *Nature* 306, 378.

Gregersen, P. K., Silver, J. & Winchester, R. J. (1987) The shared epitope hypothesis: an approach to understanding the molecular genetics of susceptibility to rheumatoid arthritis. *Arthritis Rheum.* 30, 1205.

Haworth, C., Brennan, F. M., Chantry, D., Turner, M., Maini, R. N. & Feldmann, M. (1991) Expression of granulocyte-macrophage colony-stimulating factor in rheumatoid arthritis: regulation by tumor necrosis factor-alpha. *Eur. J. Immunol.* 21, 2575.

Herzog, C., Walker, C., Pichler, W., Aeschlimann, A., Wassmer, P., Stockinger, H., Knapp, W., Rieber, P. & Muller, W. (1987) Monoclonal anti-CD4 in arthritis. *Lancet* 2, 1461.

Herzog, C., Walker, C. & Pichler, W. (1989) New therapeutic approaches in rheumatoid arthritis. *Concepts Immunopathol.* 7, 79.

Hirano, T., Matsuda, T., Turner, Miyasaka, N., Buchan, G., Tang, B., Sato, K., Shimizu, M., Maini, R. & Feldmann, M. (1988) Excessive production of interleukin 6/B cell stimulatory factor-2 in rheumatoid arthritis. *Eur. J. Immunol.* 18, 1797.

Holmdahl, R., Mo, J., Nordling, C., Larsson, P., Jansson, L., Goldschmidt, T., Andersson, M. & Klareskog, L. (1989) Collagen induced arthritis: an experimental model for rheumatoid arthritis with involvement of both DTH and immune complex mediated mechanisms. *Clin. Exp. Rheumatol.* 7, Supp, 3, S51.

Hom, J. T., Bendele, A. M. & Carlson, D. G. (1988a) In vivo administration with IL-1 accelerates the development of collagen-induced arthritis in mice. *J. Immunol.* 141, 834.

Hom, J. T., Butler, L. D., Riedl, P. E. & Bendele, A. M. (1988b) The progression of the inflammation in established collagen-induced arthritis can be altered by treatments with immunological or pharmacological agents which inhibit T cell activities. *Eur. J. Immunol.* 18, 881.

Isaacs, J. D., Watts, R. A., Hazleman, B. L., Hale, G., Keogen, M. T., Cobbold, S. P. & Waldmann, H. (1992) Humanised monoclonal antibody therapy for rheumatoid arthritis. *Lancet* 340, 748.

Johnson, B. A., Haines, G. K., Harlow, L. A. & Koch, A. E. (1993) Adhesion molecule expression in human synovial tissue. *Arthritis Rheum.* 36, 137.

Johnson, R. A., Waddelow, T. A., Caro, J., Oliff, A. & Roodman, G. D. (1989) Chronic exposure to tumour necrosis factor in vivo preferentially inhibits erythropoiesis in nude mice. *Blood* 74, 130.

Katsikis, P., Chu, C. Q., Brennan, F. M., Maini, R. N. & Feldmann, M. (1993) Immunoregulatory role of interleukin 10 (IL-10) in rheumatoid arthritis. *J. Exp. Med.* 179, 1517.

Keffer, J., Probert, L., Cazlaris, H., Georgopoulos, S., Kaslaris, E., Kioussis, D. & Kollias, G. (1991) Transgenic mice expressing human tumour necrosis factor: a predictive genetic model of arthritis. *EMBO J.* 10, 4025.

Knight, D. M., Trinh, H., Le, J., Siegel, S., Shealy, D., McDonough, D., Scallon, B., Arevalo Moore, M., Vilcek, J., Daddona, P. & Ghrayeb, J. (1993) Construction and initial characterisation of a mouse-human chimaeric anti-TNF antibody. *Mol. Immunol.* 30, 1443.

Koch, A. E., Burrows, J. C., Haines, G. K., Carlos, T. M., Harlan, J. M. & Leibovich, S. J. (1991) Immunolocalization of endothelial and leukocyte adhesion molecules in human rheumatoid and osteoarthritic synovial tissues. *Lab. Invest.* 64, 313.

Koch, A. E., Turkiewicz, W., Harlow, L. A. & Pope, R. M. (1993) Soluble E-selectin in arthritis. *Clin. Immunol. Immunopathol.* 69, 29.

Lebsack, M. E., Paul, C. C., Martindale, J. J. & Catalano, M. A. (1993) A dose- and regimen-ranging study of IL-1 receptor antagonist in patients with rheumatoid arthritis. *Arthritis Rheum.* 36 supp, S39.

Leeuwenberg, J. F., Smeets, E. F., Neefjes, J. J., Shaffer, M. A., Cinek, T., Jeunhomme, T. M., Ahern, T. J. & Buurman, W. A. (1992) E-selectin and intercellular adhesion molecule-1 are released by activated human endothelial cells in vitro. *Immunology* 77, 543.

Londei, M., Savill, C., Verhoef, A., Brennan, F. M., Leech, Z. A., Duance, V., Maini, R. N. & Feldmann, M. (1989) Persistence of collagen type II-specific T cell clones in the synovial membrane of a patient with RA. *Proc. Natl. Acad. Sci. USA* 86, 636.

Macgregor, A. J. & Silman, A. (1994) An analysis of the relative contribution of genetic and environmental factors to rheumatoid arthritis susceptibility. *Arthritis Rheum.* 37, (Suppl), S169.

Masi, A. T. (1994) Incidence of rheumatoid arthritis: Do the observed age-sex interaction patterns support a role of androgenic-anabolic steroid deficiency in its pathogenesis? *Br. J. Rheumatol.* 33, 697.

Mason, J. C., Kapahi, P. & Haskard, D. O. (1993) Detection of increased levels of circulating intercellular adhesion molecule 1 in some patients with rheumatoid arthritis but not in patients with systemic lupus erythematosus. Lack of correlation with levels of circulating vascular cell adhesion molecule 1. *Arthritis Rheum.* 36, 519.

Mauritz, N. J., Holmda
Klareskog, L. (1988)
arthritis in mice. *Art*
Miller, A., Lider, O. &
oral administration
Moissec, P., Naviliat, M.
interleukin 4 and hi
Arthritis Rheum. 33,
Moreland, L. W., Bucy,
Everson, M. P., Dad
W. J. (1993a) Use of
tory rheumatoid artl
Moreland, L. W., Margol
C. & Koopman, W.
of a phase I dose-e
Rheum. 37, supp, S2
Moreland, L. W., Sewell,
G., Woodworth, T. (
cebo-controlled pha
in patients with refr
Mosmann, T. R. & Coffn
functions of helper T
O'Hehir, R. E. & Lamb
lymphocytes by Sta
8884.
Olsen, N. J. & Cush, J.
Strand, V. & Lorenz
rheumatoid arthritis
Paliard, X., West, S. G.
Kotzin, B. L. (1991)
Science 253, 325.
Palmer, R. M. J., Hicke
Induction of nitric
Commun. 193, 398.
Panayi, G. S., Lanchbur
initiating and main
Rheum. 35, 729.
Paulus, H. E., Effer, M.
of Rheumatic Disea
arthritis patients tre
findings in patients t
Pigott, R., Dillon, L. P.,
tin, ICAM-1 and V(
tured endothelial cell
Piguet, P. F., Grau , G.
Evolution of collage
crosis factor (TNF)
510.
Pincus, T. & Callahan, L
Rheum. Dis. Clin. N.
Racke, M. K., Bonomo,

Mauritz, N. J., Holmdahl, R., Jonsson, R., Van der Meide, P. H., Scheynius, A. & Klareskog, L. (1988) Treatment with gamma-interferon triggers the onset of collagen arthritis in mice. *Arthritis Rheum.* 31, 1297.

Miller, A., Lider, O. & Weiner, H. L. (1991) Antigen-driven bystander suppression after oral administration of antigens. *J. Exp. Med.* 174, 791.

Moissec, P., Naviliat, M., d'Angeac, A. D., Sany, J. & Banchereau, J. (1990) Low levels of interleukin 4 and high levels of transforming growth factor β in rheumatoid synovitis. *Arthritis Rheum.* 33, 1180.

Moreland, L. W., Bucy, R. P., Tilden, A., Pratt, P. W., LoBuglio, A. F., Khazaeli, M., Everson, M. P., Daddona, P., Ghrayeb, J., Kilgrarriff, C., Sanders, M. E. & Koopman, W. J. (1993a) Use of a chimeric monoclonal anti-CD4 antibody in patients with refractory rheumatoid arthritis. *Arthritis Rheum.* 36, 307.

Moreland, L. W., Margolies, G. R., Heck, L. W., Saway, P. A., Jacobs, C.,Beck, C., Blosch, C. & Koopman, W. J. (1994) Soluble tumor necrosis factor receptor (sTNFR): results of a phase I dose-escalation study in patients with rheumatoid arthritis. *Arthritis Rheum.* 37, supp, S295.

Moreland, L. W., Sewell, K. L., Sullivan, W. F., Shmerling, R. H., Parker, K. C., Swatz, W. G., Woodworth, T. G., Trentham, D. E. & Koopman, W. J. (1993b) Double-blind placebo-controlled phase II trial of diphtheria-interleukin -2 fusion toxin (DAB486Il-2) in patients with refractory rheumatoid arthritis (RA). *Arthritis Rheum.* 36, S39.

Mosmann, T. R. & Coffman, R. L. (1989) Heterogeneity of cytokine secretion patterns and functions of helper T cells. *Advances in Immunology* 46, 111.

O'Hehir, R. E. & Lamb, J. R. (1990) Induction of specific clonal anergy in human T lymphocytes by Staphylococcus aureus enterotoxins. *Proc. Natl. Acad. Sci. USA* 87, 8884.

Olsen, N. J. & Cush, J. J., Lipsky, P. E., St Clair, E. W., Cannon, G., MacCune, W. J., Strand, V. & Lorenz, T. (1994) Multicenter trial of an anti-CD5 immunoconjugate in rheumatoid arthritis (RA). *Arthiritis Rheum.* 37, (Suppl) S295.

Paliard, X., West, S. G., Lafferty, J. A., Clements, J. R., Kappler, J. W., Marrack, P. & Kotzin, B. L. (1991) Evidence for the effects of a superantigen in rheumatoid arthritis. *Science* 253, 325.

Palmer, R. M. J., Hickery, M. S., Charles, I. G., Moncada, S. & Bayliss, M. T. (1993) Induction of nitric oxide synthase in human chondrocytes. *Biochem. Biophys. Res. Commun.* 193, 398.

Panayi, G. S., Lanchbury, J. S. & Kingsley, G. H. (1992) The importance of the T cell in initiating and maintaining the chronic synovitis of rheumatoid arthritis. *Arthritis Rheum.* 35, 729.

Paulus, H. E., Effer, M. J., Ward, J. R., Williams, H. J. & Coopernative Systemic Studies of Rheumatic Diseae Group (1990) Analysis of improvement in inidivial rheumatoid arthritis patients treated with disease-modifying anti-rheumatic drugs, based on the findings in patients treated with placebo. *Arthritis Rheum.* 33, 477.

Pigott, R., Dillon, L. P., Hemingway, I. H. & Gearing, A. J. (1992) Soluble forms of Eselectin, ICAM-1 and VCAM-1 are present in the supernatants of cytokine activated cultured endothelial cells. *Biochem. Biophys. Res. Commun.* 187, 584.

Piguet, P. F., Grau , G. E., Vesin, C., Loetscher, H. Gentz, R. & Lesslauer, W. (1992) Evolution of collagen arthritis in mice is arrested by treatment with anti-tumour necrosis factor (TNF) antibody or a recombinant soluble TNF receptor. *Immunology* 77, 510.

Pincus, T. & Callahan, L. F. (1993) What is the natural history of rheumatoid arthritis? *Rheum. Dis. Clin. N. A.* 19, 123.

Racke, M. K., Bonomo, A., Scott, D. E., Cannella, B., Levine, A., Raine, C. S., Shevach,

E. M. & Rocken, M. (1994) Cytokine immune deviation as a therapy for inflammatory autoimmune disease. *J. Exp. Med.* 180, 1961.

Ranges, G. E., Sriram, S. & Cooper, S. M. (1985) Prevention of type II collagen-induced arthritis by in vivo treatment with anti-L3T4. *J. Exp. Med.* 162, 1105.

Rankin, E. C., Choy, E. H. S., Kassimos, D., Soowith, M., Kingsley, G., Isenberg, D. A. & Panayi, G. S. (1994) A double blind, placebo-controlled, ascending dose trial of the recombinant humanised anti-TNFα antibody CDP571 in patients with rheumatoid arthritis (RA): A preliminary report .,*Arthritis Rheum.* 37, supp, S295.

Ronnelid, J., Lysholm, J., Engstrom-Laurent, A. & Klareskog, L. (1994) Local anti-type II collagen antibody prodution in rheumatoid arthritis synovial fluid. Evidence for an HLA-DR4-restricted IgG response. *Arthritis Rheum.* 37, 1023.

Roodman, G. D. (1987) Mechanisms of erythroid suppression in the anemia of chronic disease. *Blood cells* 18, 171.

Ruiter, D. J., Schlingemann, R. O., Rietveld, F. J. R. & Waal, R. M. W. (1989) Monoclonal antibody human endothelial antigens as vascular markers. *J. Invest Dermatol.* 93, 25S.

Saklatavala, J., Sarsfield, S. J. & Townsend, Y. (1985) Pig interleukin-1. Purification of two immunologically different leukocyte proteins that cause cartilage resorption lymphocyte activation and fever. *J. Exp. Med.* 162, 1208.

Schall, T. (1991) Biology of the cytokine RANTES family. *Cytokine* 3, 165.

Silman, A. J. & Hochberg, M. C. (1993) *Epidemiology of the Rheumatic Diseases.* Oxford University Press.

Silman, A. J., MacGregor, A. J., Thomson, W., Holligan, S., Cathy, D., Farhan, A. & Ollier, W. E. R. (1993) Twin concordance rates for rheumatoid arthritis: Results from a nationwide study. *Br. J. Rheumatol.* 32, 903.

Simon, A. K., Seipelt, E. & Sieper, J. (1994) Divergent T-cell cytokine patterns in inflammatory arthritis. *Proc. Natl Acad. Sci. USA* 91, 8562.

Stuart, J. M., Townes, A. S. & Kang, A. H. (1984) Collagen autoimmune arthritis. *Ann. Rev. Immunol.* 2, 199.

Szekanecz, Z., Haines, G. K., Lin, T. R., Harlow, L. A., Goerdt, S., Rayan, G. & Koch, A. E. (1994) Differential distribution of intercellular adhesion molecules (ICAM-1, ICAM-2, and ICAM3) and the MS-1 antigen in normal and diseased human synovia. Their possible pathogenetic and clinical significance in rheumatoid arthritis. *Arthritis Rheum.* 37, 221.

Thorbecke, G. J., Shah, R., Leu, C. H., Kuruvilla, A. P., Hardison, A. M. & Palladino, M. A. (1992) Involvement of endogeneous tumor necrosis factor α and transforming growth factor β during induction of collagen type II arthritis in mice. *Proc. Natl. Acad. Sci. USA* 89, 7375.

Trentham, D. E., Dynesius-Trentham, R. A., Orav, E. J., Combitchi, D., Lorenzo, C., Sewell, K. L., Hafler, D. A. & Weiner, H. L. (1993) Effects of oral administration of type II collagen on rheumatoid arthritis. *Science* 261, 1727.

Van den Berg, W. B., Joosten, L. A., Helsen, M. & Van de Loo, F. A. (1994) Amelioration of established murine collagen-induced arthritis with anti-IL-1 treatment. *Clin. Exp. Immunol.* 95, 237.

Van der Lubbe, P. A., Dijkmans, B. A. C., Markusse, H. M., Nassander, U. & Breedfeldt, F. C. (1994) Lack of clinical effect of CD4 monoclonal antibody therapy in early rheumatoid arthritis; a placebo-controlled trial. *Arthritis Rheum.* 37, (Supp), S294.

Vassalli, P. (1992) The pathophysiology of tumor necrosis factors. In: *Ann. Rev. Immunol.* 10, 411.

Waldmann, T. A. (1991) Monoclonal antibodies in diagnosis and therapy. *Science* 252, 1657.

Wellicome, S. M., Kapahi, P., Mason, J. C., Lebranchu, Y., Yarwood, H. & Hashard, D. O. (1993) Detection of a circulating form of vascular cell adhesion molecule-1: raised

levels in rheumatoid
92, 412.

Wendling, D., Racadot, I
by anti-interleukin 6

Williams, R. O., Feldman
ates joint disease in
9784.

Williams, R. O., Ghrayet
collagen-induced arth
anti-CD4. *Immunolo*

Williams, R. O., Mason,
CD4 and anti-TNF i
Natl. Acad. Sci. US

Wooley, P. H., Dutcher, J
human soluble tumo
induced arthritis in r

Wooley, P. H., Whalen, J.
G. (1993b) The effec
gen-induced arthritis

levels in rheumatoid arthritis and systemic lupus erythematosus. *Clin. Exp. Immunol.* 92, 412.

Wendling, D., Racadot, E. & Wijdenes, J. (1993) Treatment of severe rheumatoid arthritis by anti-interleukin 6 monoclonal antibody. *J. Rheumatol.* 20, 259.

Williams, R. O., Feldmann, M. & Maini, R. N. (1992) Anti-tumor necrosis factor ameliorates joint disease in murine collagen-induced arthritis. *Proc. Natl. Acad. Sci. USA* 89, 9784.

Williams, R. O., Ghrayeb, J., Feldmann, M. & Maini, R. N. (1995) Successful therapy of collagen-induced arthritis with TNF receptor-IgG fusion protein and combination with anti-CD4. *Immunology* 84, 433.

Williams, R. O., Mason, L. J., Feldmann, M. & Maini, R. N. (1994) Synergy between anti-CD4 and anti-TNF in the amelioration of established collagen-induced arthritis. *Proc. Natl. Acad. Sci. USA* 91, 2762.

Wooley, P. H., Dutcher, J., Widmer, M. B. & Gillis, S. (1993a) Influence of a recombinant human soluble tumour necrosis factor receptor Fc fusion protein on type II collagen-induced arthritis in mice. *J. Immunol.* 151, 6602.

Wooley, P. H., Whalen, J. D., Chapman, D. L., Berger, A. E., Richard, K. A. & Aspar, D. G. (1993b) The effect of an interleukin-1 receptor antagonist protein on type II collagen-induced arthritis and antigen-induced arthritis in mice. *Arthritis Rheum.* 36, 1305.